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BIOLOGICAL CHRONOMETRY[1]

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THE GENERAL NATURE AND SIGNIFICANCE OF THE PROBLEM

Biological chronometry, a subject that might otherwise be termed "clocks and calendars" of living things, has long been a subject of very great interest to the student of natural history. More recently, as the problem of temporal regulation of organismic behavior and all its supporting physiological processes, its fundamental importance to the study of biology as a whole has become so evident that there is a rapidly increasing number of laboratories becoming concerned with one or another facet of the problem.

Nowhere is the phenomenon of chronometry more evident than in the regularity of daily, tidal, semilunar, lunar, and annual cyclicities of animals and plants. The naturalist has watched with wonder and admiration the great precision with which numerous organisms utilize the more favorable phases of the diurnally rhythmic fluctuations in such external physical factors as light, temperature and humidity. He has similarly observed the regular tidal cycles of activities of innumerable organisms which inhabit the intertidal regions, and noted the extraordinary fortnightly, or semilunar, and synodic monthly cycles of breeding of great numbers of organisms, especially marine ones. In addition to these periodisms, there are, of course, the highly regular annual cycles of living things.

Considering all species, it is clear that the occurrence of various biological events in these cycles need bear no special relationship to the phases in the cycles of any external physical factors. Depending upon its specific biological adaptations, an organism can be maximally active at night, during the daytime, or only at dawn or at twilight. Relative to tides, maximum activity can, among the various species, clearly be at any time of tide, apparently depending upon maximum adaptive advantage to the organism. Similarly, relative to the monthly and annual cycles, each species in

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a given locality has its characteristic phase relationship to the concurrent external physical cycles. In short, since the phase relationships between organismic cycles and the physical ones can clearly, among the various species, bear any relationships whatsoever relative to one another, the only things possessed in common by them are the frequencies of the cycles and the capacities of the organisms to retain a fixed temporal relationship between the phases of any given biological cycle and the external cycles. The form of the biological cycles, that is, the specific sequence of events within the cycle, and the amount of the temporal displacement of similar biological events among the various species relative to the physical cycles are, therefore, in part or in whole clear biological contributions to the phenomenon but also themselves depend upon temporally regulated processes.

More subtle roles of solar-day and lunar-day clocks in organisms have become evident through recent investigations of animal navigation. It appears true that certain birds which may use the sun in their orientation are able continuously to correct for the changing position of the sun in the sky during the day (Kramer, 1952). Other work has indicated that an amphipod which uses the moon for orientation during its nocturnal migrations, similarly continuously corrects for the changing positions of the moon (Pardi and Papi, 1953; Papi and Pardi, 1953).

Still another role for biological chronometry became evident with the successful demonstration many years ago for certain plants that the form of the annual cycle of growth and reproduction was conditioned by the changing lengths of light periods during the year. This phenomenon, photoperiodism, which has since been shown to be widespread among organisms, provided the relatively temperature-independent mechanism needed to account for the observed regularity of the annual cycles of numerous living things. At the same time, however, it left quite unexplained the nature of the essentially temperature-independent mechanism which enabled the organism to distinguish the small differences in photoperiods. This last is obviously another of the basic problems of physiological time-measurement.

Survival values of the regular biological cyclicities are self-evident. Their contributions are manifold. Through them the organism may prepare to defend itself against the unfavorable portions of the environmental cycles or to utilize maximally for feeding and reproduction the more favorable times. Without this phenomenon, the synchronization of reproductive cycles and breeding behavior, apparently so essential to the maintenance of many species, would not exist.

A superficial consideration of biological cyclicity might lead one to an hypothesis that the observed regularity was explicable *solely* in terms of responses to well-known physical stimuli or complexes of them. However, that such an hypothesis is untenable is readily apparent simply by placing organisms in conditions constant with respect to all those factors known to influence them. The cycles may not only continue, but be even more regular than in nature. Such cyclicity, referred to as *persistent*, has been described for numerous species of organisms representing all major groups. Such

persistence has been described for many instances of solar-day, of tidal or lunar-day, and of fortnightly and monthly cycles. In fact, persistent fortnightly and monthly cycles can be simple derivatives, by periodic reinforcement, of persistent solar-day and lunar-day cyclicities. Emphasizing the remarkable precision of the persistent cycles, it has been shown that organisms in constant conditions may retain unaltered phase relationships with the external physical cycles even for periods of months.

Implicit in all earlier reports on persistent rhythmicity was the fact that the frequency of the solar and lunar cycles was temperature-independent within rather wide limits. Organisms were reported to exhibit precise solar and lunar frequencies irrespective of what constant temperature the investigator arbitrarily selected. During the past decade this temperature-independence has been firmly established through specific experimentation directed at this relationship (Brown and Webb, 1948; Pittendrigh, 1954). It is obvious, furthermore, that to be of any adaptive value to the organism, a temperature-independence of the frequencies of the cycles would be quite necessary. In fact, were this not so, persistent cyclicity would be worse than useless in the normal temperature-varying environment. Temperature-independence of a biological phenomenon to the degree required here, were the problem to be resolved solely in terms of an internal, and wholly autonomous, clock system, would pose a formidable problem indeed for the general physiologist.

The problem involves another step in its complexity. The persisting cyclicities observed in constant conditions can have their phase relations relative to the outside day-night or tidal cycles abruptly shifted by appropriate light or temperature changes by any desired degree and then retain this new relationship at least for many cycles. In other words, the phases of the persistent biological cycles even in a single species need bear no fixed relationship to external ones. This clearly dissociates, even for a single species, the phase relations of the biological cycles from any known or unknown external physical cycles. The phase relations are obviously readily resettable by well-known types of stimuli and therefore must be internally regulated. Cycles of stimuli associated with the day-night cycles, and the rise and fall of the tides, must operate *in nature* in some manner to determine these phase relations, and then in constant conditions in the laboratory a precise frequency-determining mechanism retains these phase relations constant, often apparently indefinitely.

The capacity to reset persistently the relationship of the endogenous biological cycles to the external solar-day and lunar-day ones has real value to the organism. The organism can adaptively adjust its functional patterns to the highly localized forms of the cycles of light, temperature, and humidity of its specific niche, or to the times of high and low tides on its specific beach. At the same time, however, this property, so beneficial to the organism, increases the difficulty in resolving the problem of its mechanism. For example, a rat or mouse in constant darkness may show good average 24-hour cycles of spontaneous activity. In constant low illumination, on

the other hand, the cycles may become attenuated to 25 or more hours, the apparent frequency a function, within limits, of the brightness of the illumination (Johnson, 1939; Brown, Shriner, and Ralph, 1955). It is very difficult to distinguish between an endogenous cycle being reset abruptly once in each daily cycle and one possessing a regular cycle of another frequency. Hence, it is impossible to resolve in this manner the question of whether *regular* presistent cycles even of frequencies other than those of solar and lunar frequencies could occur in the absence of concurrent external physical cycles of the natural frequencies.

A good working hypothesis for the present appears to be that there exists in organisms an endogenous clock-system which maintains its regular frequencies through some kind of an external pacemaking signal which continues to be effective under what is usually deemed "constant conditions." Such an hypothesis resolves at once certain otherwise paradoxical information. The basic frequency-determining mechanism would be presumed to retain a constant fixed relationship to the changes in the effective external physical factor. The labile biological cycles, whose phase relations are persistently alterable, may be postulated to be "plugged into" this basic clock-system at adaptively determined points. "Replugging" could be accomplished at any time by the operation of well-known kinds of "setting" stimuli, including training.

The view that there is an external pacemaker is perhaps the simplest one to account for the property of temperature-independence. There is also adequate evidence that organisms in so-called constant conditions are still receiving some signals from the external physical environment. This has been recently demonstrated in our laboratories in studies of the character of fluctuations in metabolism or in spontaneous activity in a wide variety of animals and plants kept in constant conditions (Brown, Webb, Bennett, and Sandeen 1955; Brown, Freeland, and Ralph, 1955). Highly significant correlations have been shown to exist with barometric pressure and its changes. That barometric pressure is not itself the agent is proven through finding the correlations to persist even in organisms maintained for long periods in constant pressure. Even more recently it has been learned that the amplitude of daily fluctuations in metabolism of the potato plant grown for four months under constant conditions including pressure was highly significantly correlated with the amplitude of the concurrent daily cycles of fluctuation in high-energy background radiation. In view of the existence of clear mean cycles in both barometric pressure and background radiation, it is apparent that the organism has access to external physical signals with good average cycles even under so-called constant conditions. Even if organisms utilize these mean metabolic cycles in the maintenance of the frequencies of their labile endogenous cycles, as seems reasonable to postulate, there is still little information as to the manner in which the induced metabolic cycles are so used.

There is a final problem of biological chronometry which might be mentioned in this brief introduction to the subject. To what extent do these

endogenous clocks operate under natural conditions? The role of such rhythms for organisms which emerge from burrows and other dark recesses to feed periodically, or in those which depend upon periodic migrations at appropriate times, is self-evident. In other instances, they may play the determining roles, *other environmental factors equal,* in the temporal regulation of behavior. It is tempting to speculate that these cycles are the basic physiological behavior patterns through which stimuli normally influence organismic behavior, even in direct responses to stimuli. In this latter case, this pattern would constitute a large part of the mechanism through which the response of an organism is conditioned by its recent history.

The speakers who follow me on this program will now go on to consider some special facets of the problem of biological chronometry.

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TWENTY-FOUR HOUR CYCLES IN MARINE ORGANISMS[1]

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INTRODUCTION

Periodic variations in the behavior of organisms have been observed and recorded as a conspicuous feature of our biological environment for a very long time. Indeed, such variations are often so striking that it is extremely difficult to ignore them. Thus folklore and myth abound with examples of cosmological speculation which purport to explain astronomical cycles on the basis of biological rhythms.

As plant and animal physiology emerged as experimental disciplines, some of the more striking of these cycles were studied and described. This early literature has been carefully reviewed by several authors including Welsh (1938), Kleitman (1949), Webb (1950), and Brown, Fingerman, Sandeen and Webb (1953). It quickly became apparent that there was a group of such periodic physiological variations which persisted for more or less considerable periods in conditions which were loosely described as "constant." These persistent rhythms may be contrasted with other biological cycles which are merely direct responses on the part of the organism to some environmental factor which changes in a cyclical fashion.

If we temporarily restrict our attention to cycles of 24-hour frequency as exhibited by marine organisms, we find that such cycles are very conspicuous and various. Thus persistent 24-hour cycles have been reported in light production, position of retinal pigments, color change, oxygen consumption and a variety of other parameters. Some of these cycles seem to be very broadly distributed. Thus it would appear to be the case that no marine organism which has been suitably studied has failed to manifest variations in oxygen consumption with a diurnal frequency component (see, for example, Brown, Webb, Bennett, and Sandeen, 1955).

In view of the existence of a number of recent reviews of this literature, it does not seem profitable to attempt an exhaustive listing of 24-hour cycles in marine organisms. Furthermore, the limitation with respect to frequency and habitat suggested by the title of this paper is rather arbitrary and would be an undesirable and artificial restriction to a review. Consequently, it seems preferable to review some recent contributions which have been made to the analysis of one of the most intensively studied biological cycles, the 24-hour color change rhythm of the fiddler crab, Uca.

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Following this review, the field of biological rhythms will be examined synoptically in an effort to assess the present state of our information.

THE 24-HOUR RHYTHM OF MELANOPHORE DISPERSION IN THE FIDDLER CRAB, UCA

The melanophore rhythm of the fiddler crab is a particularly convenient system for study for a variety of reasons. Fluctuations in the dispersion state of the chromatophores can be estimated accurately at short intervals without any apparent modification of the rhythm and without injury to the animals. The extent of melanophore dispersion at any given time is expressed usually as an average for the group of animals being considered and

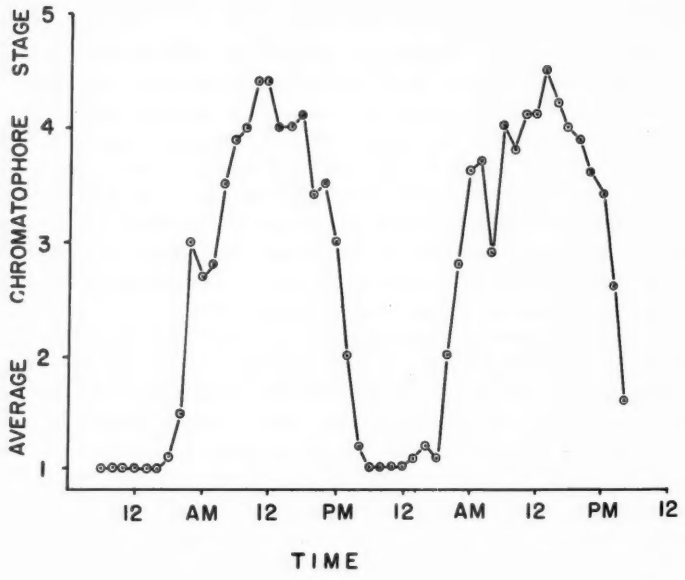

FIGURE 1. (redrawn from Brown, Fingerman, Sandeen, and Webb, 1953) Average stage of the melanophores of a group of fifty fiddler crabs maintained in darkness for the period of observation.

is estimated for each individual using the Hogben-Slome scale. In this scale, three arbitrary units are established between Stage 1 (minimum dispersion) and Stage 5 (maximum dispersion). Figure 1 is a plot of the average stage of the black chromatophores in a group of fifty animals maintained in a darkroom for forty-eight hours.

In common with a number of the rhythms described for marine organisms, the melanophore rhythm persists with a precise, 24-hour frequency for some time in constant darkness. In the case of this particular rhythm, its persist-

ence is quite striking. It has been observed in our laboratory to continue for at least two months with no demonstrable shift with respect to the solar day-night cycle. At the end of such an extended period in darkness, the amplitude of the rhythm is unimpaired. In fact, amplitude tends to increase during the first weeks in darkness (Brown and Stephens, 1951).

In view of the fact that no specific attention was paid to temperature control in the early observations of this phenomenon, its persistence for considerable periods in phase with the solar day-night cycle constitutes prima facie evidence for its independence of small variations in temperature. More precise and convincing evidence was obtained by Brown and Webb (1948a) in a set of experiments designed to test this hypothesis. Groups of animals were placed in the dark at carefully controlled temperatures and it was demonstrated that this diurnal rhythm was indeed independent of temperature at least for the range 6° C. to 26° in the sense that no deviation from the typical 24-hour frequency was detected. These workers also found evidence which suggested that at low temperatures the amplitude of the rhythm decreased. In an extension of this work, (Brown and Webb, 1948b) inhibition of the mechanism underlying the melanophore rhythm by temperatures below 6° was reported. Subsequently, Webb, Bennett, Graves, and Stephens (1953) were able to demonstrate a relationship between the time of day when animals were exposed to 5° C. and the extent of the inhibition or retardation produced.

In the course of analysis of a set of preliminary experiments, it became apparent that a sudden change of temperature might be a stimulus competent to produce a delay of the 24-hour melanophore cycle. This was the case despite the fact that animals maintained at either the upper or the lower of two temperatures used continued to exhibit a cycle of 24-hour frequency. Consequently a series of experiments was designed to investigate the phase shifts produced in the melanophore rhythm by exposure of the animals to sudden changes of temperature in the hope that the pattern of such changes might provide further information about the mechanisms underlying the cycle (Stephens, 1955a, 1955b).

SHIFTING THE PHASE

The procedure which was used to estimate the phase shift produced by the experimental treatment of the animals was as follows. Inspection of figure 1 suggests that the best estimate of the extent of a phase shift should compare the regions of greatest slope in the curve. This was taken to be stage 2.5 despite some variation in the shape of individual curves. Thus the time at which the melanophores traversed stage 2.5 as they expanded was determined for the experimental group and compared with the time of traverse in the same direction in a control group. An attempt was made to compensate for variations in shape and amplitude of the curves compared by measuring the delay of the cycle both for the morning ascending phase (Δ_u) and the evening descending phase (Δ_d). This procedure is

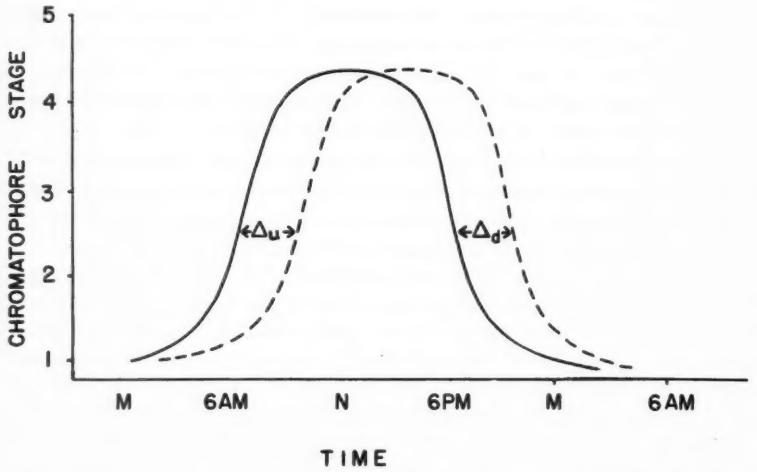

FIGURE 2. (redrawn from Stephens, 1957) Diagram of method of estimation of phase shift of the melanophore rhythm.

illustrated graphically in figure 2. At least one cycle was allowed to pass after completion of experimental manipulation of temperature before the groups were compared. At least two full cycles were followed in estimating the delay for each experimental group. Both the experimental animals and the control groups were maintained in darkness throughout the experiment. On the basis of information obtained in this fashion, the delay of the cycle could be calculated in minutes.

Temperature control was accomplished either by placing a covered pan of animals in a constant temperature room or by floating the pan on the surface of a water bath. The actual protocols employed are somewhat involved and are reproduced in detail elsewhere (Stephens, 1957). In general, it may be stated that exposure of the animals to the lowest temperature used, 9.5°C., was always effective in delaying the rhythm provided the length of the exposure was at least twelve hours.

This phase shift which was induced by exposure of the animals to low temperature exhibited a number of interesting properties. It must first be emphasized that this delay did not merely represent a slowing of the rhythm at the lower temperature since the delay was not at all related to the total length of time of the exposure. In one experiment, a 12-hour exposure to cold produced a phase shift of 3 hours and 20 minutes while an 84-hour exposure produced a phase shift of 3 hours and 29 minutes. Throughout these experiments, the magnitude of the response of the rhythm to the cold exposure appeared to be related simply to the number of times the animals were exposed and not to the length of the exposure. This was true provided the previously mentioned minimum time of 12 hours at the lower temperature was exceeded. It follows that the procedure of transfering animals from room temperature to 9.5° sufficed to delay the rhythm with respect to

a control group maintained at room temperature throughout despite the fact that the 24-hour frequency of the rhythm persisted at either temperature. Such phase shifts were stable changes in the rhythm and were observed to persist in darkness for at least ten days.

Further observation indicated that the delay induced by one exposure to low temperature summed with additional exposures separated by 12 hours at room temperature. The protocol for such an experiment is indicated graphically in figure 3 and the results plotted in the graph of figure 4. It is apparent that repeated exposures to 9.5° have each been effective in further delaying the melanophore cycle with respect to a normal control group. It may also be pointed out that the total time in the cold for Group VI which was delayed by more than 13 hours was 48 hours. This may be compared with 84 hours in the cold for Group II which exhibited a delay of approximately 3 hours. As stated earlier, the delay is apparently related to the number of exposures rather than the time in the cold.

In this particular case, the relationship between the number of exposures and the delay produced seems to be simple linear one. In general, this was repeated several times but some of the other data do not fall as naturally on a straight line so that it would be incautious to draw conclusions about the details of this relationship.

It also became apparent that the time of day at which a 12-hour exposure was begun influenced the magnitude of the observed phase shift. Thus a 12-hour exposure to low temperature initiated at 3 PM was more than twice as effective as a 12-hour exposure under the same conditions but initiated at 9 AM.

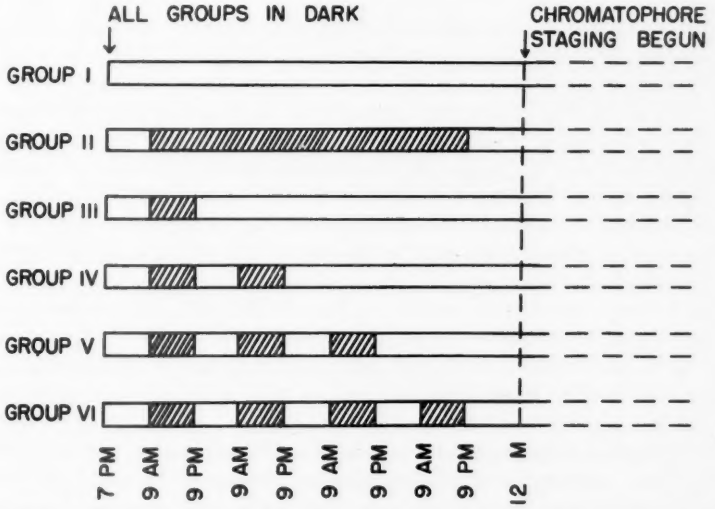

FIGURE 3. (redrawn from Stephens, 1957) Protocol for multiple exposure of experimental groups to low temperature. Shaded areas represent 9.5°, unshaded areas room temperature.

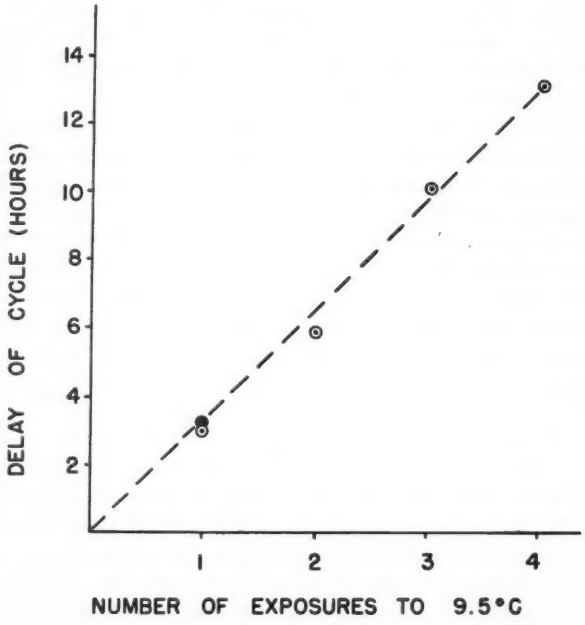

FIGURE 4. (redrawn from Stephens, 1957) Phase shift for each group in the experiment outlined in Figure 3 plotted against the number of exposures to low temperature. Solid circle is Group II.

It might appear that the effect of an exposure to low temperature was simply the result of the shock consequent on an abrupt temperature change of approximately ten degrees. However, it was demonstrated that this was not the case in two different ways. First, the transfer from room temperature to the low temperature was made slowly over a period of approximately six hours. When this was done and suitable controls observed, it was found that there was no difference in the delay produced whether the transfer was made abruptly, the change in temperature being accomplished within a few minutes, or whether it was made gradually with a maximum rate of change of two degrees per hour.

As a further experiment to rule out the possibility of the influence of the magnitude of the temperature change, animals were transfered abruptly from 31° to 18°. No effect was observed. Also there was no difference in the delay produced by transfering from 31° to 9.5° and the delay produced by transfering from 18° to 9.5°.

Summarizing these observations, we can conclude that an exposure to low temperature may be competent to induce a persistent shift of phase in the diurnal melanophore rhythm provided it fulfills the following two conditions:

1. the low temperature must lie below some threshold temperature, and
2. the low temperature must be maintained for a minimum time.

Interestingly enough, the threshold lies well above the lowest temperature which allows the persistence of the 24-hour frequency of the rhythm. It is also true that, provided the minimum time is achieved, additional time at the low temperature does not appear to increase the response. Finally, the susceptibility of the underlying mechanism to displacement by this treatment is a function of the time of day.

There is one final point with respect to this phenomenon which is of considerable interest. Unfortunately this could be observed in only a preliminary fashion. However, from observations which were made during the course of this response, it appears that the response of the first cycle may be considerably greater than the persistent phase response attained in the second and subsequent cycles. The pattern of this response is indicated in

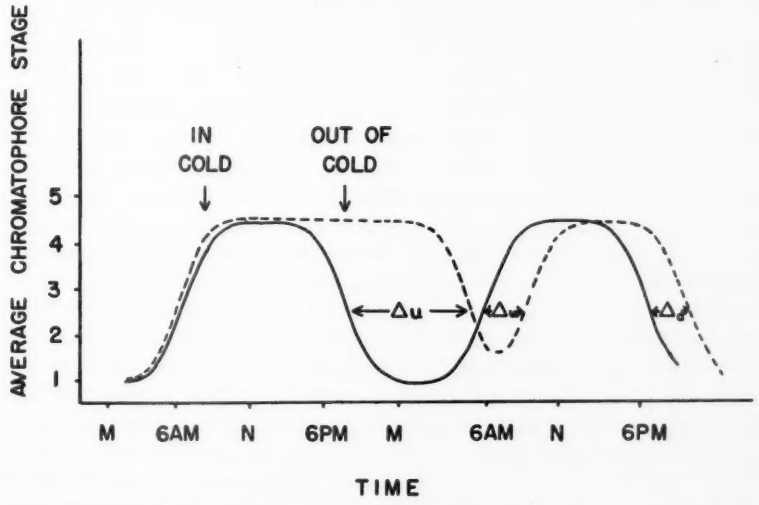

FIGURE 5. Diagram of the response of the melanophore rhythm to a 12-hour exposure to low temperature. Note the prolonged initial delay and the subsequent compensation.

idealized form in figure 5. In one case, the initial delay, Δ_d, was three times as great as the stable shift finally observed when the animals had come to equilibrium.

If this initial lability of the rhythm followed by a partial adjustment to its previous position can be verified by more extended observations, it becomes further evidence for the presence of two components in the control of this particular rhythm. Brown and Webb (1949) invoked two controlling centers to explain the responses of the melanophore rhythm to various light-dark protocols. These centers were respectively labile and readily modified by imposed environmental changes, and more stable and resistant to environmental changes. Analogous labile and stable centers were postulated by Brown and Stephens (1951) in an effort to account for the response of this

rhythm to changes in photoperiod. It is questionable whether the evidence permits distinguishing a dual mechanism from a dual response of a single mechanism. However, a labile and a stable component have been distinguished in the controlling mechanism of this rhythm by three independent investigations and this is consequently probably a real feature of this cycle.

The general pattern of response to exposure to low temperature does not seem directly comparable to responses recorded for other systems with the possible exception of Pittendrigh's data concerning the temperature independence of the clock mechanism controlling emergence in Drosophila (Pittendrigh, 1954).

INTERACTIONS BETWEEN INDIVIDUALS

Another general area of the analysis of the melanophore rhythm to which recent contributions have been made is in the study of interaction of individuals in populations. In the course of experiments involving the exposure of groups of fiddler crabs to low temperature, it was noted that the amplitude of the diurnal rhythm seemed to be sharply reduced, even when the animals were returned to room temperature. Unfortunately, the animals were not individually marked so that individual cycles could not be followed. However, an indication of the cause of the apparent decrease in amplitude was obtained by the following analysis of the available data. A complete set of data for a single cycle for a group exhibiting a low amplitude rhythm (stage 2.2 minimum to stage 2.8 maximum) was compared with similar data for a normal control group (stage 1.0 minimum to stage 4.7 maximum) which had been collected at the same time. The readings were at 90 minute intervals in each case. The percentage of the total number of readings (400 in each case) at each of the stages was calculated without regard to the time of day at which the reading had been taken. The results are listed in table 1 and the percentages for the two groups are in surprising agreement.

TABLE 1

PERCENTAGE OF TOTAL READINGS AT EACH OF THE FIVE STAGES OF THE HOGBEN-SLOME SCALE FOR A CONTROL GROUP OF NORMAL AMPLITUDE AND A LOW-AMPLITUDE EXPERIMENTAL GROUP.

Chromatophore stage	Control Animals	Experimental Animals
1	45%	47%
2	15%	18%
3	12%	13%
4	9%	7%
5	19%	15%

These results suggested that the apparent decline in amplitude observed in this group was the result of asynchrony of the persisting rhythms in individual members of the group rather than an actual decline in amplitude of the rhythm in each individual. This in turn suggested a number of interesting questions concerning the interaction or lack of interaction among the members of a population. These considerations led to the design of several experiments in the summer of 1956 to determine whether there was an interaction between individuals which played a role in the melanophore rhythm.

If such interactions are occurring in a group of fiddler crabs, brought into the laboratory and isolated in a darkroom, one might expect to be able to induce either modifications in the amplitude of the rhythm by suitable procedures, or perhaps to shift the phase the rhythm exhibited by individuals, aberrant with respect to the rest of the group.

With respect to the phase of the rhythm, observations indicate that no significant modification is effected in mixed groups. Several experiments were set up involving groups of animals composed of individuals with markedly different 24-hour rhythms. In no case did any phase shift occur. For example, in one case, thirty animals with a typical melanophore rhythm were marked and placed in the same pan with thirty animals which had been exposed to light at night and kept in darkness in the daytime. These latter were observed to have a rhythm 180° out of phase with the normal group. Groups of sixty normal animals and sixty reversed animals were kept in separate pans as controls. This experiment was followed intermittently from July 14th to August 10th. On August 10th, in the mixed group, there were only four surviving reversed animals and eleven surviving normal animals. Yet the four reversed animals exhibited a rhythm which showed no significant difference from that observed in the reversed control group and even showed the same minor peaks which characterized the control response. We may conclude then that populations do not interact under these conditions with respect to the phase of the rhythm.

Webb, Brown, Bennett, Shriner, and Brown (1956) have reported a very clear effect of population size on the amplitude of the melanophore rhythm in the fiddler crab. These investigators found that when individuals are isolated in paper cups in a low constant light intensity, the amplitude of the rhythm decreases markedly. In some cases, the melanophores may remain completely concentrated throughout the 24-hour period. Yet when animals treated in this fashion are recombined in a common container under otherwise comparable conditions, the amplitude of the rhythm rapidly returns to normal. In repeating these observations in our laboratory, we confirmed the decrease in amplitude produced by isolation of animals and further found that the amplitude returned to normal in the presence of one destalked *Uca pugilator* placed with the isolated *Uca pugnax*. The chromatophores of the destalked animal remained at Stage 1 throughout.

We may briefly summarize our information concerning the diurnal melanophore rhythm of the fiddler crab in terms of the known responses ofthis cycle with respect to amplitude, phase and frequency.

1. Amplitude
   a. completely abolished by removal of the eyestalks.
   b. affected by the proximity of other individuals.
   c. possibly decreased at low temperatures.
   d. influenced by photoperiod and time in darkness.
2. Phase
   a. can be shifted by suitable manipulation of light, temperature, and possibly other factors.
3. Frequency
   a. apparently fixed at 24 hours.

DISCUSSION

In this paper, our attention has been concentrated on the details of the 24-hour rhythm of melanophore dispersion in the fiddler crab. If we broaden our interest to include rhythms in marine organisms or even biological rhythms in general, the variety is very impressive. To remain insular for an additional moment, we need only examine the literature concerning the fiddler crab to discover a considerable variety of rhythmic phenomena. There follows a list of rhythms which have been described in the fiddler crab:

1. Diurnal rhythm of melanophore dispersion (Megušar, 1912).
2. Diurnal rhythm of chromatophore responses in eyestalkless animals (Webb, Bennett, and Brown, 1954).
3. Diurnal rhythm of susceptibility to low temperature (Webb, Bennett, Graves, and Stephens, 1953; Stephens, 1957).
4. Activity rhythm (Edwards, 1950).
5. Tidal rhythm of melanophore dispersion (Brown, Fingerman, Sandeen, and Webb, 1953).
6. Rhythms of oxygen consumption (Brown, Bennett, and Webb, 1954).

This list does not pretend to be exhaustive but is merely an example of the multiplicity of rhythms which may be found in one animal. If one extends the horizon to include marine organisms in general, the variety is greatly increased.

When confronted with variety in described phenomena, an extremely profitable preliminary activity is often a tentative classification of the phenomena in question. In this paper it is proposed to attempt a tentative taxonomy of biological rhythms in an effort to organize our information.

The title of this contribution when considered together with the other titles in this symposium might be taken to imply a set of decisions concerning basic criteria for classification of rhythmic phenomena. These criteria might be assumed to be (a) the frequency of the rhythm, (b) the habitat of the organisms, and (c) the systematic position of the organisms. In fact, this inference was not intended. In any event, this problem of a taxonomy

of rhythms is sufficiently interesting to deserve direct consideration and discussion.

CLASSIFICATION OF RHYTHMS

Let us examine the differentiae proposed in the preceding paragraph which might be applied to distinguish rhythmic phenomena one from another. Frequency might initially appear as an attractive possibility. However, we must face the unfortunate fact that many of the persistent rhythms which have been described have two or more frequency components. This would appear to be quite generally true for oxygen consumption rhythms (e.g. Brown, Freeland, and Ralph, 1955) and the tidal rhythm of melanophore dispersion in the fiddler crab is apparent as a perturbation of the 24-hour rhythm. Of course this fact does not militate against distinguishing between the diurnal and lunar components of an oxygen consumption rhythm if it is convenient to do so but in these cases there does not seem to be much evidence to support separation of the mechanism of one from the other. considering the occurence of such complex rhythms, it would appear that frequency should not be considered a basic differentiating characteristic in itself. To extend our analogy of taxonomic treatment of the subject, frequency might have specific or generic status but should not define a class.

The habitat and systematic position of organisms is an even less defensible pair of criteria. Again, Brown and his coworkers in their studies of the oxygen consumption rhythms of a variety of organisms (reviewed in Brown, 1956), have shown that there are very basic similarities between algae, flowering plants, and selected invertebrates and vertebrates. Certainly there could be no more diverse collection with respect to habitat and systematic position. Yet the oxygen consumption rhythms reported for these organisms are very closely comparable.

If we tentatively reject frequency, habitat, and systematic position as criteria which can be used to classify biological clock mechanisms, what can we suggest as a basis for our taxonomy? The simplest procedure seems to be to look briefly at all periodic phenomena in biology and see if there are not some fundamental classes we can define. If we consider phenomena as diverse as heart rates, annual breeding cycles, and long-term population fluctuations, a very fundamental distinction becomes apparent. This is the distinction between "private time" or "physiological time," and "astronomical time" or "cosmic time." Heart rate is clearly a private phenomenon dependent on the size of the animal, surface-volume ratio, circulatory efficiency, and a host of other such physiological features of the individual; it is then a biological rhythm on a physiological time scale and bears no particular relation to astronomical time. This is not to say that there might not be a diurnal rhythm of heart rate but it merely denies that the rate itself marks "cosmic time." On the other hand, seasonal breeding cycles, lunar cycles, and diurnal rhythms are significant and distinctive precisely because they are independent of just those factors which control the scale of physiological time. They are adaptively significant, as has been often

pointed out, precisely because they keep cosmic time and are not modified by the usual physiological variables.

The basic separation can then be made in considering biological rhythms between those on cosmic time and those on physiological time. This is not a new distinction. Bidder (1923) was particularly succinct in his statement that the unit of physiological time is the time required for one event in the life of the cell or the organism. On the other hand, the units in the rhythms with which we are most concerned are the chronological units; hours, days, months, and years. The problem which we are discussing may be reduced to a consideration of the mechanisms which an organism may adopt to free itself from the complete dominance of physiological time and acquire the obvious advantages of conformity to cosmic time. There seem to be at least three different mechanisms which have been adopted by organisms and these may be urged as suitable differentiae for further classification of biological rhythms.

Type I, Environment-dependent frequency. Perhaps the simplest adjustment to the exigencies of astronomical time which an organism can make is the development of a set of responses to some cyclical element in the environment. Responses to changes in light intensity are commonly adopted in marine organisms to keep the individual in an optimum position for feeding and breeding. In at least some cases, the vertical migration which characterizes many planktonic animals is purely dependent on responses to changing light intensity (Harris and Wolfe, 1955) and does not persist in constant conditions. This kind of an explanation may be suggested tentatively to account for the bewildering complexity of the fluctuations in oxygen consumption described by Brown and his coworkers. In this case the environmental stimulus has not yet been identified. However, these workers suggest that despite the standard "constant" conditions used in this work, the organisms are not being effectively shielded from some stimulus to which they respond more or less simply.

Type II, Environment-induced frequency. Somewhat more complex is the development of a control system in which cycles of activity can be induced which will persist for a few cycles in the absence of continuing reinforcement from the environment. Two examples of such systems have recently been described. Schön (1955) working with the green alga Hydrodictyon was able to show that suitable manipulation of light and darkness could induce cycles of respiration which would persist for three to five cycles in darkness. The frequency of the induced cycles could be controlled at will. The organism simply reproduces the cyclic pattern imposed on it a few times and then subsides to a more or less constant level of activity. Speaking anthropomorphically, this kind of control mechanism is based on the very reasonable assumption that tomorrow is likely to be similar to today.

The activity rhythm of the cockroach, Periplaneta, has recently been analyzed by Harker (1956) and appears to be controlled in an analogous fashion. She has not formally reported the induction of cycles other than those of a 24-hour frequency but has stated in personal communications

that this has been possible. These cycles decay after a few days in a fashion reminiscent of the behavior of Hydrodictyon. In addition, Harker has been able to localize the controlling clock in the cockroach to the subesophageal ganglia. Implantation of these ganglia taken from donors exhibiting a rhythm will induce a rhythm in decapitated animals.

Type III, environment-independent frequency. Perhaps the final step in attaining independence of physiological time is the development of a clock mechanism with a very stable and possibly genetically determined 24-hour frequency. The underlying mechanism of the melanophore rhythm of the fiddler crab would appear to be an example of such a mechanism. Although

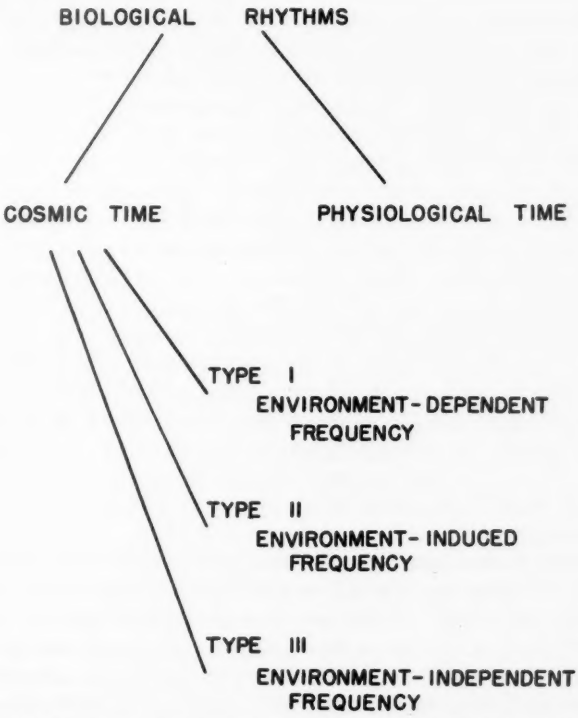

FIGURE 6. Outline of proposed classification of biological rhythms.

it has been possible to change the phase relations between the animal and the solar day-night cycle and to modify its amplitude, attempts to change the frequency of this rhythm have met with no success.

Another example of a timing mechanism with an apparently genetically determined 24-hour cycle is found in Drosophila. Brett (1955) was able to show that larvae reared in darkness could be synchronized with respect to the time of emergence by a one-minute flash of light. Effectively, administration of the light flash permitted the observation of a 24-hour cycle of

emergence the frequency of which must have been determined antecedent to this treatment.

Whether these rhythms are as completely independent of the environment as they appear to be is a difficult question to decide. It is certainly difficult to postulate mechanisms exhibiting the necessary degree of temperature independence to account for the astonishing persistence of some of these cycles which has been observed. It is also logically impossible to deny the possibility of an influence of some environmental factor so long as that factor is not specified. However, the evidence available certainly indicates that environmental timing of such rhythms, if any, is a great deal more tenuous than is the case for the types previously discussed.

The general question of the relationship between two kinds of cycles in the physiology of the same organism is clearly critical. There are a few recent studies which have dealt with this problem more or less directly. It is relevant here to consider the work concerned with transplanting organisms to a different longitude since this would presumably change most of the cyclic environmental phenomena which might conceivably be acting as periodic cues in the timing of physiological cycles. In at least one case, it has been possible to obtain evidence that the physiological cycle concerned did adjust to the change in longitude despite the maintenance of "constant" conditions. Brown (1954), studying the activity rhythm of the oyster, concluded that the tidal pattern of activity which was initially in phase with local tides at New Haven Harbor, Connecticut, gradually shifted into phase with the lunar tides at the longitude of Evanston, Illinois over a period of two or three weeks. However, the activity pattern of the oyster is essentially a pattern of respiratory activity so that it would presumably be rather directly dependent on fluctuations in oxygen consumption. Consequently, this result supplies additional evidence for the tentative classification of oxygen consumption rhythms as Type I or environment-dependent frequency phenomena.

Of perhaps more interest are the studies concerned with rhythms which might be classified as Type III or environment-independent. Rao (1954) studied the tidal rhythm of *Mytilus edulis* transplanted from its normal east coast habitat to California. He found no apparent modification of this rhythm after four weeks in California under "constant" laboratory conditions. Brown, Webb, and Bennett (1955) observed the melanophore rhythms of the fiddler crab for a six-day period in a group of animals transported from Woods Hole, Massachusetts, to Berkeley, California. Again, no modification was apparent when the behavior of these animals was compared with that of a control group maintained under parallel darkroom conditions in Woods Hole.

In attempting to interpret the results of this last study, if we assume some form of interaction between an environment-dependent cycle and the melanophore rhythm, two possibilities may be suggested. Either the melanophore rhythm changed phase with respect to some postulated environment-dependent cycle and came to occupy a new position of stability with respect

to it, or the experiment was terminated before an environmental control could express itself. The first of these interpretations is not a priori impossible but does not seem to be positively supported by the data so it must remain a possibility at the moment. However, if we accept this interpretation we must be willing to undertake to explain how the postulated environment-dependent cycle can regulate the melanophore cycle if movement of one with respect to the other is possible in this fashion. The second possibility is evident and requires no discussion. The third possible interpretation of these data would of course be that the melanophore rhythm is indeed environment-independent and that no interaction was occurring with any environment-dependent cycle.

Transplantation of animals is not the only approach to the problem of interaction between cyclical phenomena in one organism. Stephens (1956) has reported a tidal activity pattern in the mud snail, Nassa, which persists only 36 to 48 hours at room temperature. Yet after the activity is apparently quite random, a 24-hour exposure to low temperature (10°C.) is effective in regenerating the rhythm in phase with the local tides. This suggests that an underlying more persistent rhythm was made apparent by this procedure. However, it is not possible to decide what the characteristics of this underlying rhythm might be on the basis of information presently available.

It is apparent that we possess only scattered and feeble evidence concerning this critical question of the possible interrelations between various cycles in the same animal. It is in fact the burden of this tentative classification of rhythmic phenomena that in such circumstances it is better to recognize the differences which obtain between these various cycles than to obscure them by the assumption of their fundamental identity or by the assumption that one stands in a causal relation to the others.

There are other differences which might be suggested as useful in the classification of biological rhythms within the cosmic-time category. For example, another distinction which may be urged concerns the kind of phenomenon which is being timed. There would seem to be a rather basic distinction, at least formally, between the timing of an event which occurs only once in the life of the organism, and the timing of events which are repeated many times. Thus a 24-hour rhythm of division in Paramecium would seem to be generically distinct from mitotic rhythms in metazoans. Perhaps a distinction might be also made on the basis of the morphological discreteness of the timing mechanism. Opposite ends of the spectrum here would be the highly localized clock described in the cockroach by Harker (1956) which has been alluded to earlier, and the oxygen consumption rhythms of tissue slices described by Brown, Freeland, and Ralph (1955). Lack of information would seriously impair the utility of this criterion however.

In conclusion, it is hoped that the categories which have been suggested as describing and distinguishing classes of biological rhythms will be

sharpened and modified in further discussion. A preliminary classification seems to deserve discussion at this time for a variety of reasons:

1. It emphasizes the descriptive character of most of our present information.
2. It emphasizes the differences which exist between biological rhythms, even in the same organism, and may thus serve to clarify discussion.
3. It makes apparent the need for an analytic approach to the possible relationships between various types of rhythms.

It is hoped that these advantages justify the inadequacies of this attempt at such a classification.

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TWENTY-FOUR HOUR RHYTHMS OF MAMMALS IN A COLD ENVIRONMENT[1]

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The biologist accepts that some mammals, like invertebrates, may have a biological clock which keeps its physiological activity in regular periods called biological rhythms. The search for the hypothetical mammalian clock or system of clocks must continue for many years, but the activities which are regulated are more apparent. Examples of mammalian rhythms are numerous since once it is determined that an animal's locomotor activity is periodic and also controlled by inner mechanisms (endogenous), one expects to find an accompanying rhythm or cycle of heart rate, metabolism, blood sugar, blood cell count, mitosis, body temperature, and kidney function. Recently investigators using rodents have stressed two approaches, (a.) modifying rhythms or (b.) describing them in quantitative terms. For example, Holmgren and Swensson (1953) have advanced the study of the effects of reversed light cycles, while Browman (1952) has succeeded in altering the 24-hour activity rhythms of rats to one of sixteen hours. Halberg and Visscher (1956) have described quantitatively eosinophil rhythms and other rhythms in terms of a morning high and a night low which is critically dependent on the light sequence used. Calhoun (1956) has described three complexes of rodent behavior, for each of which frequency is a function of duration, and yet the duration of any behavior is completely independent of the duration of the preceeding or following one. This is referred to as an unpredictable ordering of a set of behaviors each of which has regularity in the frequency distribution of duration.

The European school now groups their quantitative work under the title of cybernetics. They suggest that work on biological rhythms be called chronobiology, and also suggest the use of a new adjective, biorhythmical. As far as their experimental work is concerned, in Europe more than in the United States, the effects on man of reversing day and night, has been analyzed on shift workers. The physiological changes which were noted, were usually due to loss of sleep, or social alterations. Apparently no large-scale controlled experiment has been done. In the area of descriptive biology related to biological rhythms it is of interest that eight-hour, rather than 24-hour rhythms have been described in insectivorous mammals (Godfrey, 1955). Finally, Brown (1956) has carried on his experiments with a different interest and has described persisting effects of exogenous

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factors superimposed upon a changing or moving endogenous rhythm of the white rat.

These are the highlights of some work in mammalian rhythms, but the work of Kleitman needs particular comment. He has continued to study the rhythms which are apparent during sleep in human subjects, and with especial profit, the rhythms apparent in infant human subjects. The application of his approach to the experiments described in the present paper will be apparent. This program also has been particularly influenced by other experiments of Kleitman (1953), in which he separated the heart rate rhythm and the body temperature rhythm of one subject. The oral temperature rhythm persisted on a 24-hour basis, after the subject's heart rate curve quickly adjusted to either an 18-hour or 28-hour schedule of rest, activity, and food intake. In other experiments he extinguished the body temperature rhythm by keeping human subjects awake for a number of days (Kleitman, 1923).

With this precedent in mind it seemed profitable to attempt to separate or extinguish some or one of the biological rhythms of rodents. Success in this endeavor would add to the understanding of a potential biological clock. For example, if a heart rhythm could be separated from a body temperature rhythm, one would postulate a separate controlling mechanism for each; if one remained linked to a rhythm of muscular activity then the supposition would be for that one, the presence of a simple dependence upon this muscular activity. Such as experimental approach would require the simultaneous measurement of several biological rhythms, a procedure which has been neglected by many intestigators. This procedure was followed with several types of mammals with appropriate attempts to separate or extinguish rhythms. A second approach was to lower or encourage the lowering of the body temperature of mammals and to record the effect upon biological rhythms. For this purpose mammals which can hibernate were used, mainly the hamster, 13-lined ground squirrel, and bat. In more detail the experimental work to be reported here consists of: (a) attempts to separate the rhythm of heart rate and body temperature in hamsters by exercise; (b) attempts to extinguish or modify by cold exposure the body temperature rhythm of rabbits, hamsters, two carnivores, ground squirrels, and bats; and (c) attempts to separate or extinguish by exposure to cold the rhythms of activity and bladder function of hamsters, and finally (d) the possible recording of rhythms during hibernation or dormancy. The latter involves a search for light and heavy phases of hibernation.

METHODS

In this program automatic recording of physiological rhythms except activity has proved unsatisfactory so far, partly because only a few animals can be recorded at one time. Therefore most of the data was obtained by observers who made readings at 7 a.m., 11 a.m., 5 p.m. and 11 p.m. In this way from ten to 35 animals at a time could be studied. For nearly all rhythms studied, an additional reading at 3 a.m. was occasionally made.

More specifically, rectal body temperatures were measured by calibrated thermocouples in metal tipped catheters, or calibrated mercury thermometers; heart rate was measured with indwelling electrodes by a Burdick Electrocardiograph; oxygen consumption was measured by a Grindeland modification of Scholander's manometric technique; total activity was recorded by tambours; and running activity by Welsh recorders. All animals, including the ground squirrels, were of known age and approximately the same age, with the exception of one species; the ages of the bats were not determined. They were all *Eptesicus fuscus.*

RHYTHMS OF HEART RATE AND BODY TEMPERATURE

As background in the attempt to measure several simultaneous rhythms in the same animals, six rhythms were described for the first time in the hamster. This species was selected because it can hibernate. Examples of five types of measurements are given in figure 1. The heart rate dropped from a mean at midnight of 412 beats/minute to 380 beats/min. at noon. This determination of the heart rate rhythm has been successfully repeated with 55 new determinations. The body temperature highs and lows are well shown as 37.5°C and 36.3°C at 11 p.m. and 11 a.m. respectively. The oxygen consumption measurements made by Richard Grindeland showed

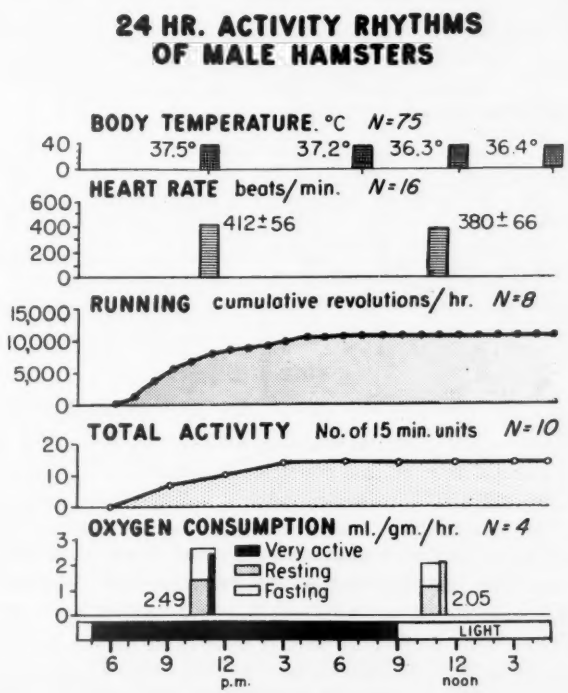

FIGURE 1.

a rhythm in spite of regular deprivation of food for twelve hours (mean values for nonfasted animals were 2.49 ml./gm./hr. at midnight and 2.05 ml./gm./hr. at noon). Looking at the profile as a whole one can see that the animal is strongly nocturnal, that the major running activity was completed by midnight, and the conspicuous locomotor activity (referred to as Type A) has been completed by 3 a.m. This means that 11 p.m. may be slightly late to be considered as the peak of physiological activity.

Attempts were next made to separate the body temperature and heart rate rhythms. It would be considered important, in the search for a clock or regulator, if one of the two rhythms could be extinguished. The experimental approach can be explained with hypothetical figures: consider that a mammal at midnight in a condition of partial muscular warm-up with a

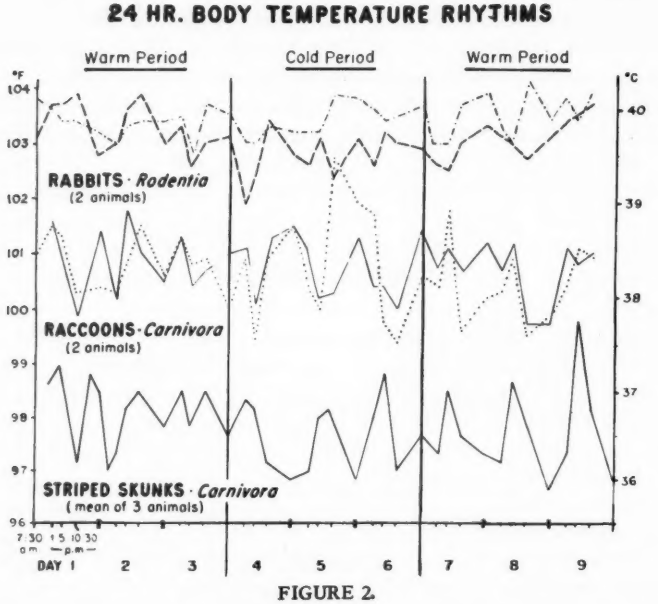

FIGURE 2.

body temperature (BT) of 37°C and a heart rate (HR) of 400 beats/min. + added exercise = *full* muscular warm-up with BT 38°C and HR 450 beats/min.; but a mammal at noon in a condition of muscular "coldness" with BT 36°C and HR 350/min. + added exercise = *full* muscular warm-up with BT 37°C and 400 beats/min. This means that the two rhythms still persist after exercise. If just the heart rate were found to be the same at noon and midnight after exercise, this rhythm would be said to be extinguished.

In actual practice using the techniques of exercising hamsters after initial measurements at 11 a.m. and 11 p.m. (Folk and Schellinger, 1954), it was found that both rhythms after exercise were essentially unaltered in amplitude and time of peak. Apparently in this animal the two rhythms are closely linked. It was now considered profitable to try cold exposure

as a means of altering or extinguishing the rhythms under study. Two species which are erroneously claimed to be hibernators were included in the study.

COLD EXPOSURE AND BODY TEMPERATURE

Body temperature rhythms were measured by identical techniques in rabbits, two pseudo-hibernators (racoons and striped skunks), hamsters, 13-lined ground squirrels, and bats. Standardized exposures were three days at 21 °C, three days at 5 °C and a return to three days of the warm temperature. The results from the three species are given in figure 2, and they show little change in cold exposure. These figures for the carnivores represent apparently the only existing basal body temperatures of these animals (mean for skunks: 36.4 °C; mean for raccoons: 38.1 °C). The data

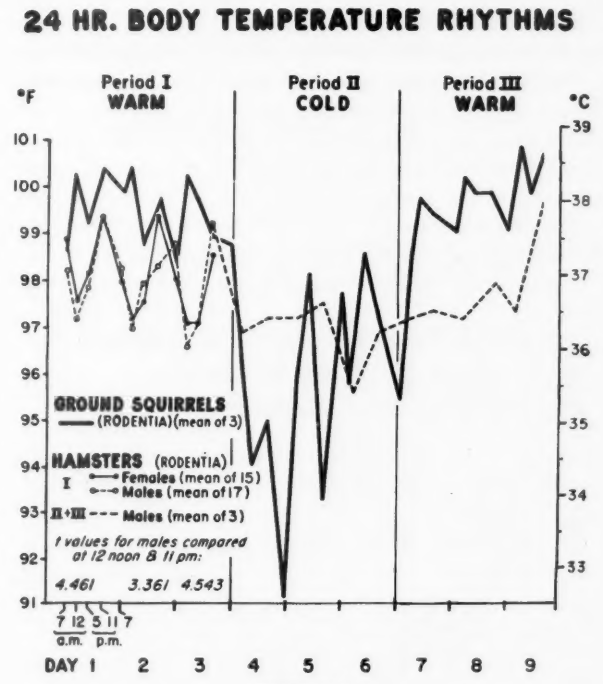

FIGURE 3.

are useful in attempting to describe the relationship between body temperature and size in carnivores. After the experiment, using constant darkness, cold exposure, and intermittent deprival of food for one month, every attempt was made to encourage a drop in body temperatures in these carnivores, but they remained stable. There was no evidence of temperature lability in these species. The data on hamsters and ground squirrels are given in figure 3. The graph for a large sample of hamsters connects with the mean

figure for three males in cold exposure and control warm-exposure. There was no appreciable effect of exposure to cold on the rhythm of body temperature of hamsters. This was not the case with ground squirrels and bats. The data for the bats are not included but they showed a non-hibernating esophageal temperature rhythm with an amplitude of over 10°C. The records for the ground squirrels deserve particular attention, because the rhythm not only had much larger amplitude in the cold and was at a lower level, but also was modified in time. The control periods show with each animal a major peak of temperature in the early morning and a minor midnight peak.

These peaks were shifted by six to ten hours by exposure to cold, an effect not demonstrated with any of the other animals except the bats. On the whole this cold-exposure test divides these animals in two groups, as far as non-hibernating body temperature rhythms are concerned; these are a temperature-labile group and a temperature-stable group. The first includes the bat and ground squirrel and the second the hamster and remaining mammals. These groupings are used in spite of the fact that about 40 percent of a colony of cold-exposed hamsters will take the "cold-vacation" path of hibernation. In contrast the ground-squirrel and bat, although different in some important respects, are alike in that they show non-hibernating temperature lability and nearly 100 percent hibernation after short exposure to cold. Also from the temperature rhythms was obtained evidence that the ground squirrel is, predominantly dayactive, although it can be active at night. Proof of this behavior pattern was so important to the study of

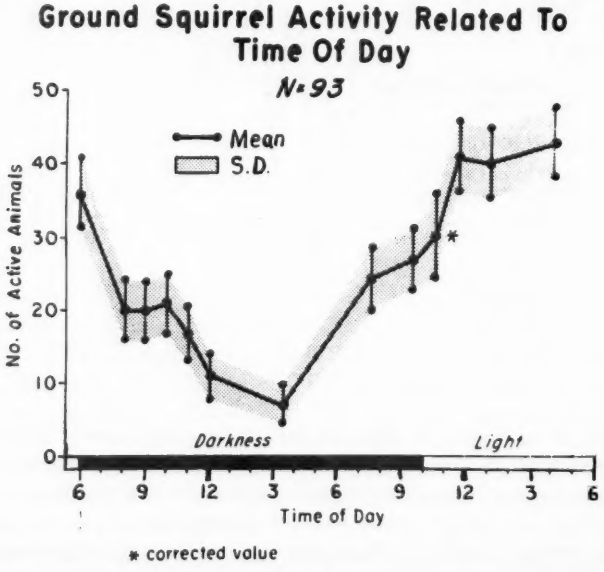

FIGURE 4.

possible rhythms during the dormancy of hibernation, that a detailed study was made. Some of the evidence is presented in figure 4. It is apparent that in a colony of 31 animals, most animals are active in daylight, although a few are active in darkness. Next the effect of cold on two rhythms simultaneously measured, rather than the single measure, will be considered.

RHYTHMS OF MUSCULAR ACTIVITY AND BLADDER FUNCTION IN COLD EXPOSURE

Attention was given to a possible rhythm of bladder function, since little consideration has been given to the possibility that this organ and the kidney act as a biological clock. A similar smooth-muscle organ, the stomach, has been implicated for many years. Bladder function and total activity of hamsters were studied simultaneously with eight recording devices for 178 days, under control conditions, in darkness and in cold. A distinct nocternal rhythm of bladder function was apparent, a rhythm which persisted in darkness. (see figure 5) This rhythm was closely linked to

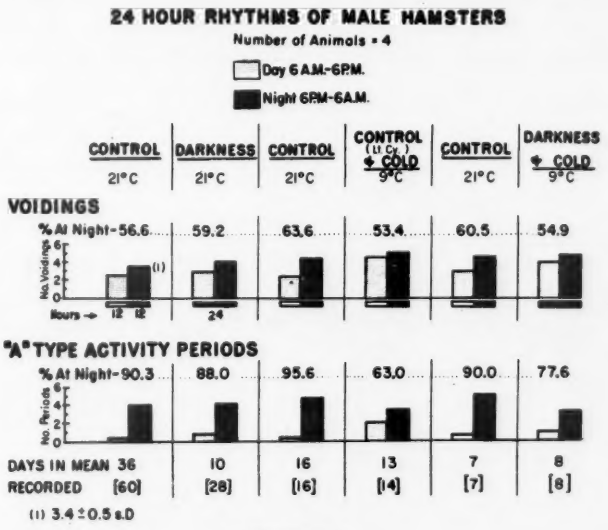

FIGURE 5.

the rhythm of conspicuous muscular activity (referred to as Type A) and at no time could the two be separated by the experimental modifications. The two measurements were altered simultaneously by exposure to cold. These experiments, which are being continued with measurements of urine volume, and with surgical modifications, will elucidate the importance of the bladder as a biological clock. They are of current interest as a contribution to the present controversy as to the extent and importance of mammalian diuresis in initial exposure to cold. The data obtained are unusual because of the homogeneity of the results from the four animals.

For example in the first control period the mean percent of voidings at night was about 57 percent. The mean number of voidings at night was 3.4, with a small standard deviation of the mean of 0.5 voidings. The percents which comprise the mean of 57 were: 58, 56, 57, 56. The 36 days were selected from 60 days of recording because only on these days did all eight recording systems work perfectly. The mean was obtained by the same selection throughout the experiment (Gault and Folk, 1957).

The study of the bladder constitutes the sixth rhythm which was shown to be conveniently measured using the hamster. With this background of observations at normal body temperature available, attention was given to a new phase-that of looking for evidence of biological rhythms at a reduced body temperature, such as in hibernation. Would a 24-hour rhythm be extinguished, or prolonged to some time such as two to four days, or would it persist in the cold? Such experiments on rhythms at a cold temperature were sparked by those of Welsh using crayfish and Brown, et al. (1954) which suggest the presence in invertebrates of a biological process independent of temperature. As yet our measurements of oxygen consumption of hibernating mammals are not accurate enough to use to measure a possible rhythm. However, many other observations were made during hibernation, of not only the hamster, but also the ground squirrel and the bat.

EXPERIMENTS TO RECORD PERSISTING RHYTHMS IN HIBERNATION

Hamsters and ground squirrels were marked in hibernation with sawdust, and maintained in isolation without known stimuli from the external environ-

FIGURE 6: Hibernating Groundsquirrels. Note how sawdust on the fur can indicate awakening and activity.

ment except for eight hours of light per day. (see figure 6) This light effect was retained so that there would be only one change from control

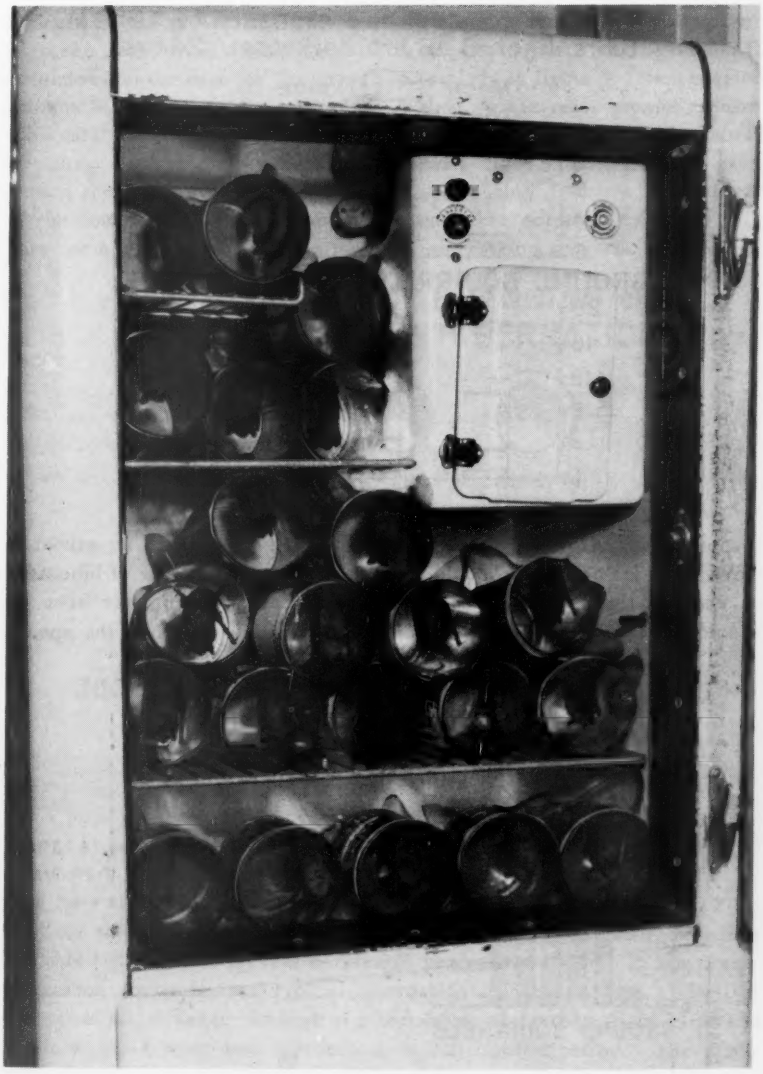

FIGURE 7: Bats arranged in refrigerator for study of light and deep hibernation.

conditions, that of exposure to cold. Details of the methods used have been described earlier (Folk and Farrand, 1956). The bats on the other hand were maintained in constant darkness in a refrigerator where they could be easily observed. (see figure 7) The toenails and toe-holds were

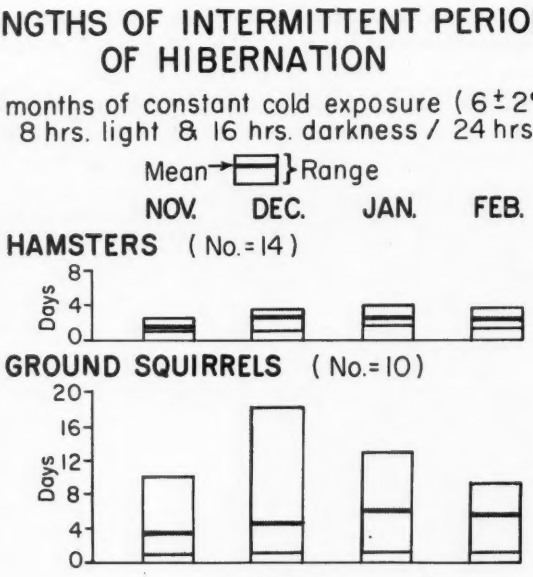

FIGURE 8.

touched with yellow oil paint, so that it was evident when an animal had moved from its perch. It should be emphasized that the type of hibernation in each of these three animals is quite different. This has been well elucidated by Farrand, et al. (1956). Considering just two of the species,

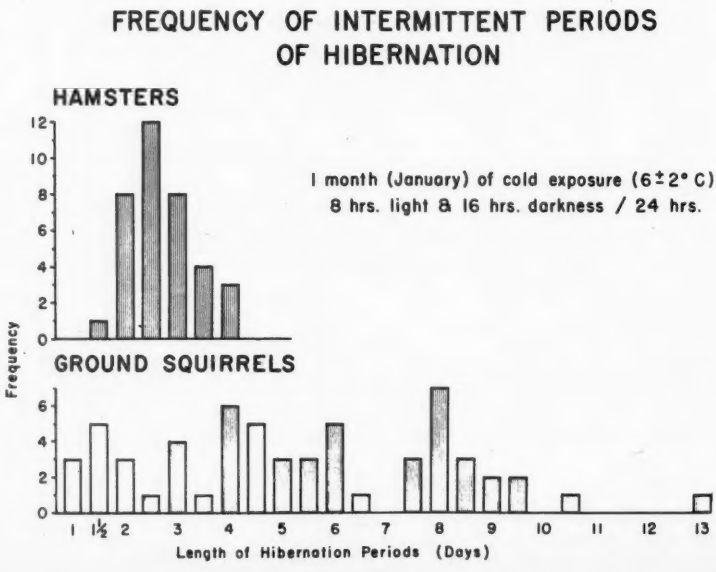

FIGURE 9.

one notes that the mean duration of intermittent hibernation of hamsters in the fall was 1.6 days increasing to 2.7 days by January. For ground squirrels the periods were longer, a mean of 3.2 days increasing to 5.9 days. Sometimes the ground squirrels would stay in hibernation from twelve to eighteen days, while the hamsters never more than four. (see figure 8) The breakdown of the data for January alone is given in figure 9. Most hamsters hibernate for two to three days at a time, while most ground squirrels hibernate from four to eight days. The observation technique consisted of checking each animal without disturbance, three times each 24 hours. During the periods of hibernation one may probably discount any influence of the observer or the light which was on in the coldroom each day. The data for beginning and awakening from hibernation is given in figure 10.

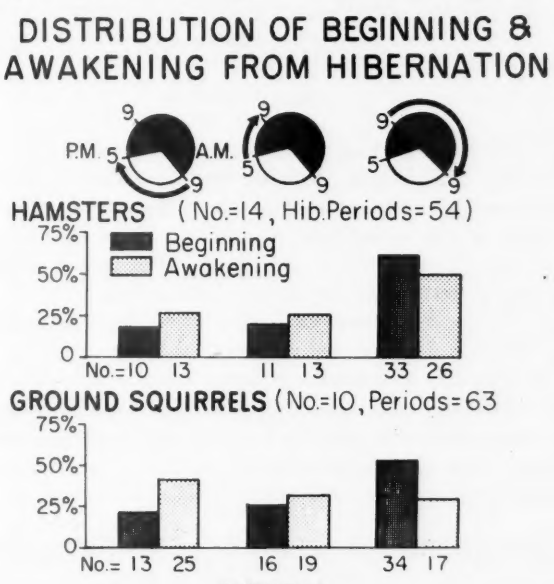

FIGURE 10.

Note here that the light, from tungsten bulbs giving approximately 7 foot candles, was on in the cold room from 9 a.m. to 5 p.m. It is of interest that this light did not prevent nearly 100 percent hibernation in the case of the ground squirrels, and typical hibernation in the hamsters. The light did appear to influence the time of hibernation, since only about 23 percent of the animals went into hibernation in the lighted period. The ground squirrels and hamsters awoke from hibernation at somewhat different times, a fact that suggests that unknown exteroceptive factors did not influence the experiment. The data from the ground squirrels is of particular interest. Animals awoke on 17 occasions from 9 p.m. to 9 a.m., in 19 cases they awoke from 5 p.m. to 9 p.m., and in 25 cases, they awoke from 9 a.m.

HIBERNATION INDEX FOR BATS

NUMERICAL UNIT	
1	DEEP HIBERNATION
2	HEAD RESPONSE: Ears or head move slightly once, at puff of air.
3	BODY RESPONSE: Single movement of whole body.
4	QUIVERING: Response by quivering and usually chittering call. Goes back to quiescent state.
5	LOCOMOTOR ACTIVITY: Due to stimulus or spontaneous Moves feet or wings or moves from vertical to horizontal position.

FIGURE 11.

to 5 p.m. Predicted awakening at random for the same times would be 32, 11, and 22. The data provide evidence that a biological clock or mechanism has influenced the awakening process so that these animals awake in daylight, which is their usual time of activity. These results encourage a repetition of this time-consuming experiment. The data from the hamsters follows a different pattern and one equally interesting.

The experiments on the bats consisted of ten days of frequent observations and 75 days of occasional observations, with all specimens marked so movement could be observed. In these animals five stages of hibernation were apparent. (see figure 11) They are: deep hibernation, a head response, a body response, a quivering response, and locomotor activity. The first three units are arbitrarily designated as hibernation, and the last two (four and five) as the semi-dormant condition. It was an unexpected

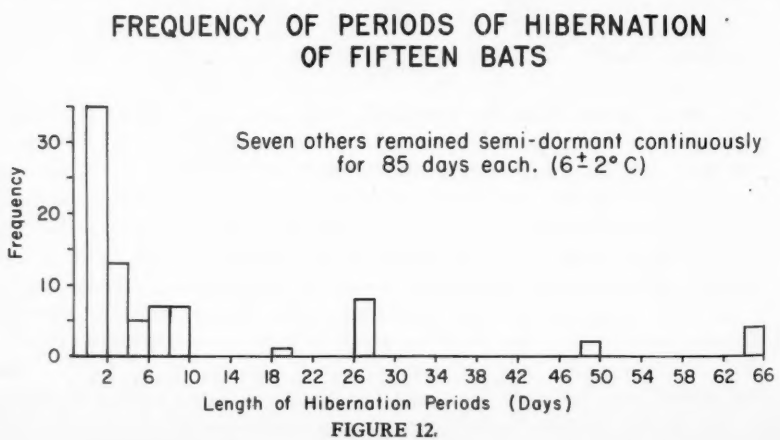

FIGURE 12.

observation that seven of the specimens were observed only in stages four or five; thus it is doubtful that they ever reached the state of deep hibernation. Most specimens remained in a dormant condition for from two to four days, and then moved around. (see figure 12) Some did not move for 50 or 60 days. The purpose of the observations was to determine whether there is a rhythm of awakening and activity in those animals which did become dormant. The mean hibernating index for 22 specimens was obtained at random times for ten days. This would mean that if the index unit came out to be one, then all specimens would be in deep hibernation. If it was five, all specimens would be active. The data are plotted in figure 13. The results show an equal amount of activity in solar day and

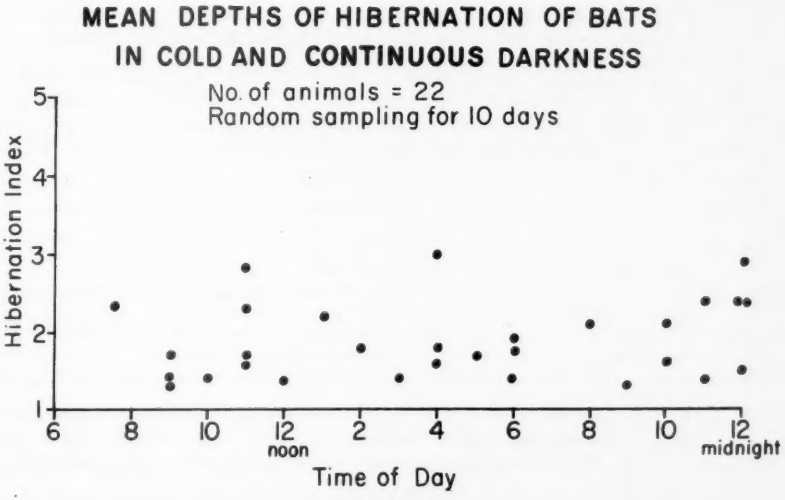

FIGURE 13.

solar night. The results at first appear to confirm those of Hock (1951), who could not show a 24-hour rhythm in the oxygen consumption of hibernating bats. However, when the data are graphed separately by the day, there appears to be a 48-hour rather than a 24-hour rhythm. The mean for seven odd days at 11 p.m. is 2.2, and for the even days is 1.9. This approach is promising but much more data will be needed.

ACKNOWLEDGEMENTS

Large contributions of time and interest were made toward obtaining the results described here, by Mary A. Folk, Richard L. Farrand, Mona R. Gault, and Richard Grindeland.

SUMMARY

These experiments have consisted of systematic attempts to separate or extinguish several types of biological rhythms, as has been done in man,

only this time using exercise and cold exposure. A second phase was to determine whether natural hypothermia (i.e., hibernation) would succeed in extinguishing the biological rhythms present at normal body temperature. The results provide further evidence of the remarkable fixity and non-modifiability of most rodent biological rhythms. This was demonstrated in data which showed the close linkage in rodents of the rhythms of bladder function and muscular activity, and of heart rate and body temperature. In the hibernation experiments some evidence was obtained for regularity in awakening of dormant animals. Light and deep forms of hibernation are described.

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LUNAR RHYTHMICITY IN MARINE ORGANISMS[1]

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Numerous marine organisms carry on rhythmical activities correlated with the phases of the moon and times of high and low tides. Such rhythms typically involve either locomotor activity, reproductive activity, oxygen consumption, color change, or a combination of these. Lunar rhythms have a frequency of 29.5 days, the time required for the moon to make one revolution around the earth. Tidal rhythms have a frequency of 14.8 days or 12.4 hours. Rhythms with a frequency of 14.8 days are also referred to as semilunar. Fourteen and eight-tenths days is the average time between two successive low tides or high tides that occur at the same time of day on a beach with semidiurnal tides or between a high tide and a low tide that occur at the same time of day on a beach with diurnal tides. Twelve and four-tenths hours is the interval between two low or two high tides in a region of semilunar tides (e.g. Woods Hole, Massachusetts) or between successive high and low tides in a region of diurnal tides (e.g. Ocean Springs, Mississippi).

In addition to diurnal and semidiurnal variations, the amplitude of the tidal variation is influenced by the phases of the moon. When the sun and moon are in conjunction, the tidal amplitude is maximal (spring tides); when the sun and moon are in opposition the tidal amplitude is minimal (neap tides). Spring tides occur at 14.8 day intervals near the times of new and full moon. Neap tides occur about the first and last quarters of the moon's phases. However, the exact relationship between the phases of the moon and the time of spring and neap tides depends upon the topography of the region under consideration.

LUNAR RHYTHMS OF MOTION AND LOCOMOTION

The first report of a persistent tidal rhythm exhibited by an organism was presented by Bohn (1903) and Gamble and Keeble (1903, 1904) for the flatworm *Convoluta roscoffensis*. Specimens were observed to emerge onto the surface of the sand at times of low tide and to disappear again into the sand as the tide rose. This rhythmic behavior persisted in laboratory aquaria free from direct contact with the tides. Presumably during the daytime the Convoluta came to the surface at times of low tide to allow the

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symbiotic algae in the digestive tract to take advantage of the increased light intensity for purposes of photosynthesis. The Convoluta would then retreat into the sand as the water rose in order to avoid the wave action of the incoming tide.

Soon after the initial description of the tidal rhythm of Convoluta, rhythms with a tidal frequency were found in several other forms. Bohn (1904) reported that the polychaete *Hedistes diversicolor* exhibited a persistent tidal rhythm that involved emergence of the annelid from the sand as the tide rose. He also observed that the snail *Littorina rudis*, normally an inhabitant of the higher regions of the intertidal zone and consequently only covered by water during the spring high tides, became active once every 15 days when maintained under constant conditions in the laboratory. The periods of activity coincided closely with the occurrence of spring tides.

Bohn and Piéron (1906) described a tidal rhythm of expansion and contraction of the sea anemone *Actinia equina*. The anemone expanded at times of high tide and retracted when exposed to air by the falling tide. Bohn (1906, 1907, and 1910) demonstrated that the tidal rhythm of Actinia was endogenous since the rhythm persisted for three to eight days under constant conditions in a laboratory aquarium where the anemones were always under water. Bohn and Piéron (1906) and Piéron (1906, 1908a, b) claimed that the tidal movements of *Actinia equina* were performed a little in advance of the actual tidal changes, thus giving evidence of what might be called an anticipatory reaction.

Drzewina (1907) observed a persistent tidal rhythm of phototaxis in the hermit crab *Clibanarius misanthropus* collected in an area of considerable tidal amplitude. The hermit crabs were negatively phototactic at times of low tide and positive at times of high tide. Crabs of the same species collected in a region where the tidal variation was slight did not exhibit a tidal rhythm of phototaxis. Wheeler and Brown (1936) observed a lunar periodic swarming of the prawn *Anchistioides antiguensis* at times when the moon waned from last quarter to the first quarter of the new cycle.

Stephens, Sandeen, and Webb (1953) described a persistent tidal rhythm of locomotor activity of the mud snail, *Nassa obsoleta*. At times of low tide relatively few snails exhibited spontaneous locomotor activity whereas activity was maximal at times of high tide. Snails collected from localities with different tidal times showed activity patterns correlated with the times of low and high tides in their original habitat.

Brown (1954) described a tidal cycle of opening and closing of the valves of the oyster *Crassostrea virginica*. The valves of Crassostrea tended to be open at times of high tide more than at other times of the tidal cycle. The Crassostrea were collected in Long Island Sound and shipped approximately 1000 miles westward to Evanston, Illinois. For a number of days after the arrival of the oysters in Evanston the tidal maxima of openness of the valves were synchronized with the times of actual high tide in the original habitat of the oysters. The tidal maxima thereafter gradually

shifted to coincide with the times of maximal lunar gravitational attraction in Evanston.

Bennett (1954) continuously recorded the opening and closing of the valves of the common quahog, *Venus mercenaria*. Analysis of the activity records revealed a persistent tidal rhythm of opening and closing. The times of minimal openness corresponded closely with the times of low tide in the area where the quahogs were collected. A lunar cycle of openness was also evident. Major peaks of openness occurred every 29 days and a minor peak occurred about midway in the cycle.

Rao (1954) demonstrated an endogenous tidal rhythm in the rates of water propulsion of the mussels *Mytilus californianus* and *Mytilus edulis*. Periods of greater pumping activity coincided with the times of high tide in the area where the specimens were collected. The tidal rhythm was independent of temperature between 9°C. and 20°C. and persisted in the laboratory in phase with the tidal cycle of their original habitat for at least a month in constant light, constant darkness, and under natural day-night conditions.

Mytilus edulis were shipped from Woods Hole, Massachusetts to Los Angeles, California, where their tidal rhythm was out of phase with the local tidal cycle. Some of the *Mytilus edulis* were placed in the intertidal zone for one week. During this period the activity pattern of the *Mytilus edulis* shifted to coincide with the times of the local tides. Although this rhythm is persistent and endogenous at least in part, its phases evidently can be reset in synchrony with the natural rhythms of a new enviroment.

LUNAR RHYTHMS OF BREEDING

An excellent review of lunar and tidal rhythms of breeding in marine organisms has been published by Korringa (1947). He described in detail his own observations of lunar periodicity in breeding of the larviperous oyster *Ostrea edulis*. The number of oyster larvae per unit volume of sea water was determined daily from the middle of June through late August annually from 1937 through 1946. When *Ostrea edulis* spawns, the eggs are brought into the maternal mantle chamber where they develop into larvae. Eight days after spawning the larvae swarm. Korringa found swarming was not limited to a few days each year but was distributed over several weeks with primary and secondary peaks of swarming activity. A swarming rhythm obviously depends directly upon a periodicity of spawning. The large majority of the maxima of swarming occurred about 10 days after a full or new moon. Therefore, spawning must be maximal at the times of spring tides that occur about two days after full and new moon in the Basin of the Osterschelde, Holland, where Korringa made these observations. So long as the water temperature was greater than 15°C., temperature was of little importance in determining spawning time or swarming of the larvae. Every breeding season showed one great maximum in swarming that occurred between June 26 and July 10 about 10 days after the full or new moon that occurred during this period. Korringa postulated that rhythmical differences in water pres-

sure due to alternating spring and neap tides were the mediating factor in the control of spawning.

Several other instances of lunar and tidal rhythms of breeding activity have been reported. Lysaght (1941) reported that more egg capsules of the gastropod *Littorina neritoides* were found in plankton tows made during spring tides at Plymouth, England, than at other times during the breeding season of Littorina.

Fage and Legendre (1923a, b, 1927), Legendre (1925), and Fage (1933) have described the nuptial dance of the polychaete *Platynereis dumerili.* This annelid breeds from May to September between sunset and midnight during the first and third quarters of the moon.

Potts (1913) and Fraser (1915) described the breeding rhythmicity of the "Fire-worm", *Odontosyllis phosphorea,* whose breeding season lasts from the end of July until the beginning of October at Departure Bay, British Columbia. The nuptial dance occurs at sunset and lasts for 30 to 60 minutes at the first and last quarters of the moon. The luminescence of the swarming females attracts the males.

In contrast to the semilunar rhythm of breeding activity of *Odontosyllis phosphorea, Odontosyllis enopla* has a lunar rhythm, breeding in Bermuda during the last quarter of the moon only (Galloway and Welch, 1911). The luminescent females swarm shortly after sunset during the last quarter of the moon in July and August.

The precise swarming of the Palolo worm, *Eunice viridis,* near the Samoan and Fiji Islands has attracted the attention of several investigators. Predominant among these were Collin (1897), Friedländer (1898, 1899a, b, 1904), Krämer (1899a, b,), Woodworth (1903, 1907), and Gravier (1924). A clear-cut swarming occurs at the last quarter of the moon from October to January and is probably due to rhythmical gamete maturation. Eunice is a nocturnal organism. Consequently, moonlight probably plays a greater role than sunlight in gamete maturation. At Upolu in the Samoan Islands, Eunice swarms when a low tide occurs shortly before sunrise during the last quarter of the moon. All worms reach sexual maturity about the same day of the year. At the proper phases of the moon and tide they cast off the epitocous portion of their body that then swims to the surface where breeding occurs.

The grunion, *Leurestbes tenuis,* is probably the most widely-known organism possessing a lunar rhythm (Thompson, 1919; Clark, 1925). The grunion deposits its eggs in the sand of California beaches near the high water line during spring tides. Spawning occurs from March to midsummer with a peak of activity in April and May. The grunion spawns at night one to three days after a full or new moon during the hour after the water has reached its highest level. The eggs then develop unmolested by wave action until two weeks later when the high tides of the next spring tide expose the eggs and the fish hatch. Clark (1925) demonstrated that the same individuals take part in reproductive activity every spring tide and suggested that rhythmical gamete ripening is correlated with the tidal sequence. Egg maturation appeared to require 15 days.

LUNAR RHYTHMS OF OXYGEN CONSUMPTION

Persistent rhythms of oxygen consumption with tidal, semilunar, and lunar frequencies have been described in a variety of marine organisms. Gompel (1937) made hourly determinations of the oxygen consumption of a coelenterate, *Actinia equina*, an echinoderm, *Paracentrotus lividus*, an annelid, *Arenicola marina*, three molluscs, *Patella vulgata*, *Haliotis tuberculata*, *Mytilus edulis*, a crustacean, *Xantho floridus*, and two fishes, *Rhombus maximus* and *Pleuronectus platessa*. A general persistent rhythm paralleling the tidal changes was evident in each species examined. Maximal oxygen consumption occurred a little before the time of high tide, the minimum a little before low tide.

A persistent tidal rhythm of oxygen consumption of the gastropod *Urosalpinx cinereus* was described by Sandeen, Stephens, and Brown (1954). The minimal rate of oxygen consumption occurred about five hours after low tide and the maximal rate occurred about two to three hours before low tide.

The most detailed information concerning rhythms of oxygen consumption has been obtained with the fiddler crabs *Uca pugilator* and *Uca pugnax* by Brown, Bennett, and Webb (1954), Brown, Sandeen, and Ralph (1954), and Brown, Webb, Bennett, and Sandeen (1955). In addition to a 24-hour rhythm of oxygen consumption, both species have tidal, semilunar, and lunar rhythms of metabolic rate. The tidal component of oxygen consumption provided a minimum rate about the time of high tide in the habitat where the crabs were collected and a maximum shortly before the time of low tide. A semilunar rhythm was also apparent as a result of the periodic reinforcement of the 24-hour and tidal rhythms for only once every 14.8 days were these two rhythms in synchrony with one another.

Fiddler crabs kept in the laboratory gradually shifted the phases of their tidal rhythm from a minimum about the time of high tide to a minimum about the time of lunar zenith and nadir that in this instance was six hours out of phase with the time of high tide in the original habitat of the crabs. The form of the daily variation in oxygen consumption of the fiddler crabs *Uca pugnax* and *Uca pugilator* was also shown to exhibit a monthly variation; the form of the rhythm for a two week period straddling a new moon was different from the form for a two week period straddling a full moon.

The rate of oxygen consumption of *Uca pugilator* whose eyestalks had been removed was also determined. These eyestalkless *Uca pugilator* exhibited a 24-hour rhythm of oxygen consumption but lacked a tidal rhythm. The absence of a tidal rhythm of oxygen consumption in eyestalkless *Uca pugilator* may be due directly to removal of the X-organ and sinus gland. Rhythmical secretion from these endocrine sources may control the tidal rhythm of oxygen consumption in intact specimens.

The hourly values in rate of respiration of both species of Uca showed a significant correlation with the concurrent rate of barometric pressure change. The rate of oxygen consumption increased in a direct relationship with the concurrent rate of barometric pressure fall and decreased in a direct relation-

ship with the rate of pressure rise. A lunar rhythm of oxygen consumption is in all probability imposed upon an endogenous lunar rhythm of oxygen consumption.

LUNAR RHYTHMS OF COLOR CHANGE

Persistent tidal and semilunar rhythms of color change have been described in three species of fiddler crabs, *Uca pugnax, Uca pugilator,* and *Uca speciosa,* and in the blue crab, *Callinectes sapidus,* by Brown, Fingerman, Sandeen, and Webb (1953) and Fingerman (1955, 1956). The first description of persistent tidal and semilunar rhythms of color change was presented by Brown, Fingerman, Sandeen, and Webb (1953) for the fiddler crab, *Uca pugnax,* collected near Woods Hole, Massachusetts, where the tides are semidiurnal. The crabs darkened by day and lightened by night in accordance with their 24-hour rhythm of color change. In addition to the 24-hour rhythm a tidal rhythm appeared to be progressing across the 24-hour rhythm at the rate of 48.8 minutes per day as evidenced by a skewing of the 24-hour rhythm curve as the time of low tide progressed into the afternoon. The tidal rhythm was evidenced by a supplementary dispersion of the chromatophores one to three hours prior to the time of low tide.

In order to obtain further information concerning the tidal rhythm, fiddler crabs were collected at Chapoquoit near Woods Hole and placed in a darkroom where the average chromatophore stage of 50 animals was determined each day hourly from 5 A.M. to 10 P.M. The curves depicting the daily excursion of the chromatophores showed a tidal rhythm was superimposed upon the 24-hour rhythm and was progressing across the 24-hour rhythm at the proper tidal frequency. When low tide occurred early in the morning the curve was skewed to the left and when low tide occurred in the afternoon the curve was skewed to the right. The curve was symmetrical about noon when low tide occurred about 10 A.M. The curve was bimodal when low tide occurred sufficiently early in the morning so that the low tide in the morning and the low tide in the evening, 12.4 hours later, could both produce a supplementary dispersion of the melanin superimposed upon the 24-hour pigmentary excursion.

In order to determine the precise rate at which the tidal cycle progressed over the 24-hour cycle the data were treated as follows. The 18 hourly periods of observation of a single day were divided into six periods of three hours duration. The sum of the average chromatophore stages of each of the six periods was then divided by the sum of the 18 average chromatophore stages obtained during the day. Each quotient was then expressed as a percentage. The percentage for any three hour period corresponding most closely with the time of low tide on a particular day would be near a maximum for the percentages for that particular time of day and the percentage for a three hour period farthest away from the times of low tide would be close to the minimum for that time of day.

Both the maximal and minimal percentages passed across the six periods of the day at a rate closely approximating that at which a tidal cycle is ex-

pected to progress. The curves for each of the six portions of the day exhibited a 14.8 day cycle, the average expected interval between days on which low tides occur at the same time of day on a beach with semidiumal tides. As a result of possessing rhythms with both 12.4 and 24.0 hour cycles the crabs also possess a 14.8 day cycle, the interval between days on which these two rhythms repeat similar time relations to one another. No loss of synchrony of the tidal rhythm of color change of fiddler crabs in the laboratory with animals on the beach still subject to the action of the rhythmic tidal changes was evident.

In order to determine whether the phases of the tidal rhythm are set by the tidal changes within the native habitat or are directly determined by the phases of the moon and only secondarily correlated with tides, the tidal rhythms of fiddler crabs collected in regions with different tidal times were compared. Chapoquoit on Cape Cod and Lagoon Pond on Martha's Vineyard were selected as the sites for collection of the Uca because low tide occurred about four hours later in the day at Lagoon Pond than at Chapoquoit. In other words, low tide at any given hour of the day would occur at Chapoquoit about five days later than at Lagoon Pond, the number of days required for the tidal cycle to progress about four hours over the 24-hour cycle. The phases of the tidal rhythm of the Uca from Lagoon Pond averaged 4.9 days earlier than the rhythm of Chapoquoit Uca. On the basis of a tidal rate of 48.8 minutes per day, 4.9 days is equivalent to four hours, the tidal difference between Lagoon Pond and Chapoquoit. Evidently, the phases of the tidal rhythm of *Uca pugnax* are directly determined by the local tidal situation and once these phases are set, they continue unchanged under constant laboratory conditions.

Another series of experiments was performed in an attempt to determine whether the two rhythmic mechanisms, 24-hour and tidal, were completely independent of one another in the organism or were, on the other hand, in some way associated with one another. As described above, the two rhythms may bear different relationships to one another depending upon the local tidal siutation. But once the relationship has been set and the animals have been removed from the tidal scene, can the 24-hour rhythm be shifted in phase without altering the phases of the tidal? In order to solve this problem Uca were collected in one area and divided into two lots. One lot, the control, was placed in constant darkness. The phases of the 24-hour rhythm of the second group were shifted abruptly backwards by three consecutive midnight-to-6-A.M. periods of illumination. Analysis of the 24-hour and tidal rhythms of both groups of Uca revealed that the 24-hour rhythm had been shifted backward 4.9 hours and the tidal rhythm 4.6 hours. The tidal rhythm, therefore, appears to be functionally associated with the 24-hour rhythm because shifting the latter produces corresponding shifts in the phases of the tidal rhythm.

The melanophores of the legs of *Uca pugnax* tend to concentrate their pigment after removal from fiddler crabs in the day phase of their 24-hour rhythm (Hines, 1954). The degree of melanin dispersion determined 30 min-

utes after autotomy reflected both a 24-hour and a tidal rhythm. During the day phases of the 24-hour rhythm when the melanin of intact Uca was dispersed, relatively little concentration occurred after autotomy near the time of low tide. An increased degree of concentration was observed when legs were isolated near the time of high tide during the day-time. The endogenous tidal rhythm of color change of *Uca pugnax* is temperature-independent between 13°C. and 30°C. (Brown, Webb, Bennett, and Sandeen, 1954).

Persistent tidal and semilunar rhythms of color change have been described in the blue crab, *Callinectes sapidus,* by Fingerman (1955). The rhythms were similar to those described by Brown, Fingerman, Sandeen, and Webb (1953) for the fiddler crab *Uca pugnax.* However, the Callinectes were collected in a region of diurnal tides (Lake Pontchartrain, Louisiana) whereas the *Uca pugnax* were collected in a region of semidiurnal tides (Woods Hole, Massachusetts). The tidal rhythm of Callinectes had a 12.4 hour frequency just as the tidal rhythm of *Uca pugnax.* However, the time between successive low tides in a region with diurnal tides is 24.8 hours. Evidently the center of tidal rhythmicity in Callinectes operates solely on the basis of tides spaced 12.4 hours apart, independent of the nature of the tides, high or low. The tidal rhythm of *Uca pugnax* is timed to exert its maximal effect about times of low tide whereas the Callinectes showed no difference between their tidally rhythmical responses at times of high and low tides.

During approximately three days of every tidal cycle in a region of diurnal tides the tides become semidiurnal when the moon is in the plane of the equator. This change from diurnal to semidiurnal tides had no effect upon the tidal rhythms of the blue crabs under observation in the laboratory indicating that the rhythm is a deep-seated phenomenon and is not readily influenced by atmospheric or enviromental conditions such as lunar gravitational changes.

Tidal rhythms of color change have also been observed in two species of fiddler crabs in addition to *Uca pugnax. Uca pugilator* and *Uca speciosa* were collected on the beach at Ocean Springs, Mississippi, where the tides are diurnal (Fingerman, 1956). The tidal rhythms of both species were similar in nature to the tidal rhythms of *Uca pugnax* and *Callinectes sapidus.* Both the *Uca pugilator* and *Uca speciosa* were collected from a restricted portion of the beach. Analysis of the tidal rhythms of both species revealed that the *Uca speciosa* behaved as if low tide occurred for them 7.5 hours earlier in the day than low tide for the *Uca pugilator.*

Inspection of the beach at Ocean Springs revealed that the burrows of *Uca pugilator* were found only in the sand of the open beach whereas the burrows of *Uca speciosa* were found only in the marsh grass from the high tide mark to the open sand. Rarely did a *Uca speciosa* venture out of the marsh grass onto the open sand at low tide. On the other hand, at low tide the *Uca pugilator* left their burrows and moved to the water's edge to feed.

In order to explain the tidal difference between the two species of Uca the following hypothesis was developed. As the water begins to recede

following a high tide, in effect a local low tide occurs earlier for the *Uca speciosa* in their burrows among the marsh grass than for the *Uca pugilator* in their burrows in the open beach. The *Uca speciosa* would, therefore, be free to leave their burrows and feed earlier than the species living in the sand, thus accounting for their tidal maxima occurring 7.5 hours earlier in the day than the tidal maxima of the *Uca pugilator.*

In order to test this hypothesis measurements were made during low tide of the portion of the beach where both species were collected. The width of the beach from high tide to low tide marks was 205 feet. The distance from the high tide mark to the first *Uca pugilator* burrows in the sand was 80 feet. Twelve and four-tenths hours are required for the water to recede from the high tide to the low tide mark. The time required for the water to recede the 80 feet from the first *Uca speciosa* burrows to the first *Uca Pugilator* burrows was then calculated. The value is 4.9 hours and is quite close to the result observed in the laboratory for the tidal difference between the two species. The phases of the tidal rhythm are apparently set by the tides within an extremely limited strip of beach.

The sums of the hourly average chromatophore indices obtained on each day of observation of *Uca speciosa* and *Uca pugilator* revealed a semilunar rhythm of amplitude of pigment dispersion. The rhythm was more evident in *Uca pugilator* than in *Uca speciosa.* The semilunar rhythms of amplitude of color change of *Uca speciosa* and *Uca pugilator* were out of phase with one another by the same time as were their tidal rhythms of color change.

Recently the hypothesis presented by Fingerman (1956) that the phase difference of the tidal rhythm of *Uca pugilator* and *Uca speciosa* at Ocean Springs, Miss., was due to the distance of their burrows from the high tide mark was tested (Fingerman, unpublished data). Specimens of *Uca pugilator* were collected at Ocean Springs, Miss., from two isolated groups of burrows that were different distances from the high tide mark. Calculations revealed that the receding water reached the lower burrows 1.6 hours after it began to uncover the set of burrows closer to the high tide line. This difference in hours is equivalent to two days in a tidal cycle. Observation of the color changes of both groups of *Uca pugilator* in the laboratory revealed a two day difference in their tidal rhythms. The Uca from the burrows closer to the low tide mark behaved as if low tide occurred for them 1.6 hours later in the day than for the fiddler crabs from the burrows higher on the beach.

ADAPTIVE SIGNIFICANCE

The tidal rhythms of fiddler crabs are of adaptive value because the fiddler crabs leave their burrows to feed at times of low tide. Uca from a beach with semidiurnal tides only emerge from their burrows to feed when low tide occurs during the day-time, whereas Uca from a locale with diurnal tides feed when low tide occurs during the day-time or night-time. If Uca from a habitat with diurnal tides behaved as Uca from a region of semidiurnal tides and fed only when low tide occurred during the day-time, then Uca

from a region of diurnal tides would not feed for about ten consecutive days when low tide occurred during the night-time.

The fact that the phases of the tidal rhythm of color change of Uca living at different levels of a beach appear to be set by the time of local low tide at the site of their burrows indicates that the tidal rhythm may have a greater adaptive significance than previously anticipated.

The significance of the tidal rhythm of color change of the blue crab, *Callinectes sapidus*, is not immediately evident since this species is permanently aquatic and does not appear to depend upon low tide to feed as does Uca. The semilunar rhythm of color change of Callinectes may be more important in the life of the organism than the tidal rhythm.

Lunar and tidal rhythms are readily brought into synchrony with external physically varying events, indicating further the highly adaptive character of these rhythms. The *Uca pugilator* and *Uca speciosa* used in the investigation of Fingerman (1956) moved into the portion of the beach where they were collected less than a year prior to their collection. In all probability their tidal rhythms were reset by the tides where their burrows were located within that period of time. The phases of the tidal rhythm of pumping of Mytilus can also be set in phase with environmental rhythms (Rao, 1954).

Temperature-independence is another characteristic that indicates the highly adaptive character of lunar and tidal rhythms. Independence of temperature is a necessity in poikilotherms for otherwise their rhythms would constantly be getting out of phase with their environmental rhythms. If the latter situation were to occur the organism would probably be better adapted if it had no rhythm at all.

POSSIBLE MECHANISMS

Lunar rhythms observed in the laboratory are probably the resultant of an endogenous component and an exogenous environmental rhythm. Evidence for an endogenous component of a lunar rhythm has been presented for the fiddler crab *Uca pugnax* by Brown, Webb, and Bennett (1955). Uca were transported from Woods Hole, Mass., to Berkeley, California, within a 24-hour period. When data from Uca in California were compared with the data from Uca still in Woods Hole, there appeared to be no tendency for the cycles of the crabs in California to drift away from the controls in Woods Hole. The crabs were able to mark off quite accurately periods of solar and lunar day-lengths. Rao (1954) showed a similar endogenous mechanism in Mytilus shipped from Woods Hole to Los Angeles. The tidal rhythm of the mussels shifted only after the animals were exposed to the California tides.

An exogenous component of rhythmicity was shown by Brown, Webb, Bennett, and Sandeen (1955) in their experiments on rhythms of oxygen consumption of fiddler crabs. The rhythms showed a significant correlation with barometric pressure changes. This exogenous component of rhythmicity may function to keep the endogenous component in phase with environmental rhythms. The imposed exogenous rhythm may in some manner contribute to the temperature-independence of the internal center of rhythmicity.

The fact that the tidal rhythms of crabs from habitats of diurnal and semidiurnal tides are similar indicates that the frequency of 12.4 hours was of primary significance in the evolution of these rhythms and not the phenomenon exhibiting the particular frequency, that is high and low tides.

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ENDOGENOUS RHYTHMS IN INSECTS AND MICROORGANISMS[1] †

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INTRODUCTION

Twenty-four hour rhythms in organisms may be considered to be the rule rather than the exception. It is likewise the rule that such rhythms persist under constant conditions of light and temperature in the laboratory. This feature led sometime ago to their description as "persistent" rhythms or "endogenous" rhythms. The latter phrase carries the implication that the observed periodicity of organic activity is independent of those "Residual Periodic Variables," (cf. Pittendrigh, 1957; Pittendrigh and Bruce, 1957) such as: air pressure, cosmic ray intensity, etc., that remain uncontrolled in the usual laboratory analysis. There is a further implication in describing persistent rhythms as endogenous: the observed periodicity is taken to reflect the organisms's capacity to measure absolute time. Thus, in recent years thinking about endogenous rhythms has become oriented by the deduction that organisms possess clocks as part of their overall adaptive organization.

The subject of endogenous rhythms in insects and microorganisms is too extensive to be reviewed comprehensively in a paper of the present scope, and we have accordingly restricted our discussion to one of several possible approaches to the subject. Specifically, we have deliberately set aside consideration of their functional or adaptive meaning; our ultimate aim is the elucidation of the mechanism of biological clocks and the present paper restricts itself to examining the insect and microorganism data from this viewpoint.

The selection of organisms as diverse as insects and microorganisms for joint discussion merits comment. It bears directly on an essential feature of the writers' approach to the problem of clocks which is deliberately comparative (Pittendrigh, 1957; Pittendrigh and Bruce, 1957). It is our working hypothesis that biological clocks, in general, share a common fundamental mechanism that evolved early in the history of cellular organisms. The aim of our work at Princeton is to test this hypothesis. It has been adopted for two broad reasons: first, it seems to us as evolutionary biologists, to be intrinsically both attractive and plausible; and, second, if it withstands experimental test, it constitutes the most promising line of approach to the detailed causal analysis of the clock mechanism. For

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if biological clocks do possess a common mechanism, the student is free to be guided in his choice of experimental material by the sole criterion of technical suitability.

There is, however, a further merit to the comparative approach which seems to us to make it essential at this stage in the study of living clocks. The experimenter is currently restricted to assaying the behavior of biological clocks only through their effects on the physiological systems they control. Thus, he measures periodicities of locomotor activity, of color change, of eclosion frequency, or of phototactic response, etc. So long as he restricts himself to a single organism, he has no obvious way of knowing whether the observed response to experimental manipulation, such as light and temperature stimulation, is a function of the clock mechanism itself or the unknown physiological "black boxes" which lie between the clock and the assayed system like locomotor activity. In other words we need to know, in approaching the problem of clock mechanisms, which features of the rhythmic system are special to an individual species and which features are common to most or all species.

As one of us has noted earlier (Pittendrigh, 1957) there is no *a priori* probability that our hypothesis will prove correct; the occurrence in different species of alternate causal mechanisms to a common functional system is a biological generalization. But our hypothesis is at least clear and testable, and is sustained by two recent results which in our opinion are of first rate importance for the field as a whole. The first of these is the demonstration by Rawson (1956) that in homeotherms (deermouse) the endogenous rhythmic system is as temperature independent as that of other classes, even when the usual mammalian homeothermy is experimentally broken down. This is indicated in the published discussion following the paper by Pittendrigh, (1957). There can be no doubt following this observation that the basic elements of the clocks in mammals must have evolved in and been derived from poikilotherms in which natural selection for temperature independence of the clock as such could operate. The second observation sustaining the idea of a common and early evolutionary origin to biological clocks is that of Bruce and Pittendrigh, (1956) that major features of the more familiar metazoan clocks are present even in Euglena, a unicellular organism.

TOWARDS A MODEL FOR BIOLOGICAL CLOCKS

Pittendrigh (1957) has laid out explicitly the arguments supporting the conclusion that persistent daily rhythms are purely endogenous in origin. The most important evidence, as he notes, is the fact that most such rhythms have a natural period which, though close to, is not exactly that of a solar day. The exact value of the natural period may depend slightly on the specific value of the temperature or the light intensity and may vary from one individual to another. However, the existence in "constant conditions" of an endogenous rhythm with a natural period which is only slightly modifiable by the specific conditions, and which is not at all determined by the preceding conditions, is considered to be an essential feature of the clock.

TABLE 1

RHYTHMS IN INSECTS

Ecological (and other) Studies of Rhythms in Insects Illustrating Their Great Diversity and Extensive Occurrence

Type of Rhythm	Insect Studied	Comments	Investigation
Eclosion or Emergence	Drosophila	Many other more recent studies have also been made of the eclosion rhythm in Drosophila.	Bremer, 1926 Bünning, 1935 Kalmus, 1935
	Clunio marinus (midge)	Field rhythm studies of a semi-lunar rhythm which is very probably clock controlled.	Caspers, 1953
	Cecidomyidae (gall midge)		Barnes, 1930
	Chironomids (midges)		Palmen, 1955
	Scopeuma stercoraria (dung fly)		Lewis & Bletchley, 1943
Activity	Drosophila		Roberts, 1956a
	Dixippus morosus (stick insect)	Rhythm continuing in DD for 21 days. Also investigated egg laying and defecation rhythms and did some hormone studies.	Kalmus, 1937–1938
	Orthoptera (cricket)	Rhythm persisting for two weeks in DD with a suggestion of a tendency for an unstable reset.	Lutz, 1932
	Passalus cornutus (beetle)	Rhythm not observed in DD	Park, 1937
	Periplaneta americana (cockroach)	Many other studies have been made using cockroaches.	Cloudsley-Thompson, 1953a
	Blatta orientalis (cockroach)	One such study is referred to in Table 2.	Gunn, 1940
	Ptinus tectus Boie (spider beetle)	Loss of rhythm in LL, but in LL with a temperature cycle rhythm was regained. Suggestion of an unstable reset.	Bentley, Gunn, and Ewer, 1941
Time sense	Bee	A great many studies have been done using bees and only a few references will be given here.	Beling, 1929 Kalmus, 1933 Stein-Beling, 1935
		Orientation and navigation.	von Frisch, 1953
	Ants and termites	The claim is made that a rhythm is learned. See, however, Reichle (1942–44). Grabensberger also investigated the effect of drugs.	Grabensberger, 1933–34
Other types	*Photinus pyralis* (firefly)	Investigations of a rhythm in the frequency of light flashing.	Rau, 1932 Buck, 1937
	Tenebrio molitor (mealworm beetle)	Behavior rhythm	Cloudsley-Thompson, 1953b
	Dytiscus fasciventris (water beetle)	A rhythm of pigment migration and of bioelectric potential in the eye is investigated.	Jahn & Wulff, 1941 and 1943

TABLE 2

RHYTHMS IN INSECTS

Particular Features of the Biological Clocks Which Have Been Studied in One or More of the Insects

Natural period close to but different from 24 hours	Drosophila	Eclosion	At 16° C, eclosion in DD occurs with a 24.5-hour period—Pittendrigh, 1954. In constant light eclosion may occur with a shortened period—Pittendrigh and Weiss, 1956.
	Drosophila	Activity	The period of several individual adults has been measured and are definitely less than 24 hours in DD—Roberts, 1956b.
	Byrostria fumigata	Activity	The period of one individual has been measured as more than 24 hours—Roberts, 1956b.
	Leucophaea maderae	Activity	The period of one individual has been measured as less than 24 hours—Roberts, 1956b.
Temperature independent but temperature sensitive	Bee	Time sense	At temperatures ranging from 16° to 32°, and with 8° temperature changes, Wahl was not able to show any modification of the "time sense"—Wahl, 1932.
	Drosophila	Eclosion	Demonstration of sensitivity to temperature changes but essentially temperature independent period between 16° C and 26° C—Pittendrigh, 1954.
Entrainment	*Pseudosmittia arenia* (midge)	Hatching	Entrainment to LD cycles of equal light and dark with period lengths from 12 hours to 48 hours. Also, frequency demultiplication effects observed. In some cases a 12-hour cycle results in a 24-hour rhythm—Remmert, 1955.
	Ptinus tectus Boie (spider beetle)	Activity	A rhythm lost in LL is reestablished by a 24-hour temperature cycle—Bentley, Gunn, and Ewer, 1941.
	Drosophila	Eclosion	Simultaneous 24-hour cycles of LD and temperature (with varying phase relationship) suggest stronger effect of LD cycle and also suggest fixed phase relationship between entraining cycle and entrained rhythm. Entrainment to temperature cycle in DD—Pittendrigh, 1957.
Single stimulus initiation and resetting	Drosophila	Eclosion	Single stimulus initiation—Bünning, 1935; Kalmus, 1938; Pittendrigh, 1954; and Brett, 1955.
	Drosophila	Eclosion	Single stimulus resetting by light and temperature—Pittendrigh, 1954.
	Drosophila	Activity	Single stimulus resetting by light—Roberts, 1956b.
	Photinus pyralis (firefly)	Light flashing	Single stimulus resetting by light—Buck, 1937.
	Ephestia (meal worm)		Rhythm reset by temperature—Scott, 1936.

TABLE 2 (*continued*)

Transients	Drosophila	Eclosion	Transients due to temperature changes were observed by Kalmus (1940a) and Pittendrigh (1954), and transients due to light stimuli were noted by Pittendrigh (1954) and interpreted as transients—Pittendrigh, 1957.
	Dixippus morosus (stick insect)	Activity	Transients due to temperature drop observed—Kalmus, 1937–38.
Conditions for persistence or loss of rhythms	*Dixippus morosus* (stick insect)	Activity	Loss of rhythm in continuous light—Kalmus, 1937–38.
	Ptinus tectus Boie (spider beetle)	Activity	Loss of rhythm in continuous light—Bentley, Gunn, and Ewer, 1941
	Pseudosmittia arenia	Hatching	Loss of rhythm in continuous light—Remmert, 1955.
	Pseudosmittia arenia	Hatching	Loss of rhythm in continuous dark—Remmert, 1955.
	Drosophila	Eclosion	Persistence in either LL or DD—Pittendrigh and Hooper, 1954.
	Drosophila	Activity	Persistence in either LL or DD—Roberts, 1956b.
Other considerations	Drosophila	Eclosion	Anoxia experiments demonstrating stopping of the clock—Kalmus, 1938; Pittendrigh, 1954.
	Bee	Time sense	Ether-narcosis experiments failed to affect the time sense but extreme cold (5°–7°C) did introduce a delay—Kalmus, 1933.
	Periplaneta americana (cockroach)	Activity	Physical localization of the clock by transfer of the rhythm when transferring the sub-oesophageal ganglion from a rhythmical donor roach to a non-rhythmic roach—Harker, 1956.
	Dacus (Strumeta) tryoni (fruit-fly)	Eclosion (Ecdysis)	A persistent rhythm continuing in LL or DD can be induced during the adult stage of the previous generation or during the larval stage but light treatment during the pupal stage seems to be without influence on the phase of the rhythm.

These general features of the biological clock, together with many other detailed features and characteristics suggest that it is appropriate to consider an endogenous self-sustained oscillator with a characteristic natural period as a model for the biological clocks[3]. The synchronization in nature of field rhythms to precisely a 24-hour period by virtue of the daily cycles of light and temperature is thus thought of as entrainment of the endogenous oscillator by these variables. Similarly, effects such as the apparent initiation of, or resetting of the phase of rhythms by single stimuli

[3]Kalmus (1935) in using the term "Eizenfrequenz" was clearly approaching a line of thought similar to that being developed by the present writers: Pittendrigh and Bruce (1957), Pittendrigh (1957). In later papers, however, Kalmus (1940 b) pursues a very distinct line of thought in his gramaphone model which is an abandonment of the oscillator model he was earlier approaching and which we are concerned to develop here.

as well as transient effects are thought of in terms of a self-sustained oscillator.

ENDOGENOUS RHYTHMS IN INSECTS

The universality and diversity of the endogenous rhythms in insects is illustrated by tables 1 and 2 which are not comprehensive but are sufficiently extensive to illustrate the point that endogenous rhythms are very common in all types of insects. Many of these investigations have centered on adaptive and ecological considerations and relatively few are concerned primarily with elucidating the mechanism of the clock. Such observations as have been made, however, support, and in no way contradict, the general point of view of the nature and significance of the biological clock set out above. In fact, the general model of the clock as an endogenous oscillator outlined in the preceding scetion was initially suggested to us by the properties of insect clocks and that of Drosophila in particular. We consider the following specific features of endogenous rhythms in insects to be of general significance with respect to the clock mechanism:

The demonstration of a natural period (NP) close to, but not exactly equal to 24 hours. While this important feature of endogenous rhythms is currently best supported by studies on mammals and microorganisms, there are nevertheless three clear cases in insects. Eclosion in Drosophila at 16° C has a period in constant dark of about 24.5 hours (Pittendrigh, 1954), and eclosion in constant light may have a period which deviates even more from 24 hours (Pittendrigh and Weiss, 1956). The activity rhythm in adult Drosophila as well as in the cockroaches *Byrostria fumigata* and *Leucophaea maderae* is also measurably different from 24 hours (Roberts, 1956 b).

Temperature sensitive but temperature independent. This feature of the clock may by implication be considered to be generally true, since the periods of all rhythms studied are close to 24 hours independently of the arbitrary temperature at which the investigations were made. In the case of the bee, Wahl (1932) was not able to demonstrate any dependence of the time-sense of the bee on temperature differences or temperature changes. Eclosion in Drosophila occurs with a periodicity which has a Q_{10} of the order of 1.02 (Pittendrigh, 1954). The characteristic temperature sensitivity also is well illustrated by these Drosophila studies. (We now reject the idea of a distinct terminal clock (Pittendrigh, 1954) for reasons based primarily on unpublished work.)

Entrainability. The synchronization of field rhythms in nature to the 24-hour cycles of light and temperature was attributed to entrainment. Entrainment has, however, been demonstrated more directly, and not only, to cycles of a 24-hour period. The experiments of Remmert (1955) on hatching in the midge Pseudosmittia are an example. Besides demonstrating entrainment to different periods, Remmert has demonstrated fre-

quency demultiplication by a factor of one-half. Entrainment of an otherwise non-rhythmic organism by a temperature cycle alone has been accomplished using the spider beetle *Ptinus tectus Boie* (Bentley, Gunn, & Ewer, 1941) and Drosophila (Pittendrigh, 1957). Entrainment of Drosophila eclosion to light and temperature cycles of 24-hour period but different phase relationships suggests that light has a stronger effect than temperature (Pittendrigh, 1957).

Single stimuli initiation. The initiation of a rhythm by means of a single stimulus of light or temperature was shown by Kalmus (1938), Pittendrigh (1954), and Brett (1955) to occur in Drosophila eclosion. It has been pointed out by Pittendrigh (1957) that two alternative interpretations of experiments of this type are possible. One interpretation postulates that the non-rhythmic population has not had their clocks set in motion and is non-rhythmic for this reason and the single stimulus "initiates" the population rhythm by initiating clock motion simultaneously in all individuals. The other interpretation postulates that the clocks in a non-rhythmic population are all running but are out of phase with each other. The single stimulus "resets" and synchronizes the rhythm in all individuals to give an apparent "initiation" of a rhythm in the population. In our present discussion we shall use the word "initiation" to apply to either interpretation.

Resetting with single stimuli. The use of single stimuli either to start a rhythm in a non-rhythmic system (initiation), or to change the phase of an existing overt rhythm (resetting), has only recently been adopted as a possible tool for investigating the properties of the clock. The results with Drosophila (Pittendrigh, 1954) suggest that both the steady-state, and the transient, response to such stimuli will prove to be useful information.

Conditions for the persistence of, or loss of, rhythms. It is customary to use the terminology LD to represent a periodic cycle of light and dark. If 12 hours of light are followed by 12 hours of *dark,* one writes LD 12:*12.* If continuous light is used, one writes LL; and for continuous darkness DD. The idea that persistent rhythms are more frequently lost in LL than in DD has some support in the insects. Dixippus (Kalmus, 1937–1938), *Ptinus tectus* Boie (Bentley, Gunn, & Ewer, 1941), Pseudosmittia (Remmert, 1955), and undoubtedly many other insects lose their rhythms in continuous light. One, namely Pseudosmittia, loses its overt rhythm also in the dark; however, there are reasons to believe the rhythm to be endogenous. On the other hand, some rhythms like Drosophila eclosion, can have a persistent rhythm both in LL and DD.

Other considerations. Early experiments designed to demonstrate the endogenous nature of 24-hour rhythms included efforts to stop the clock temporarily by anoxia, and similar means. Experiments suggesting that anoxia can cause a delay in the rhythm should be mentioned in this connection (Kalmus, 1938; Pittendrigh, 1954).

We should not leave the subject of insect rhythms without mentioning the experiments of Harker (1956), who has shown that, at least in the case

TABLE 3

RHYTHMS IN MICROORGANISMS AND SIMPLE PLANTS

Natural period close to but different from 24 hours	Euglena	Phototaxis	Bruce and Pittendrigh, 1956
	Oedogonium	Sporulation	Buhnemann, 1955a, b, c, d.
	Pilobolus	Sporulation	Schmidle, 1951 (See Figure 10)
	Gonyaulax	Bioluminescence	Hastings and Sweeney, 1956
Temperature independent but temperature sensitive	Euglena	Phototaxis	Period ranges from 23.3 hours at 33° to 26 hours at 16.7°C. Temperature sensitivity is also noted—Bruce and Pittendrigh, 1956.
	Oedogonium	Sporulation	Period ranges from 20 hours at 17.5°C to 25.5 hours at 27.5°C. Temperature coefficient is thus negative. Temperature sensitivity is also noted—Buhnemann, 1955c.
	Pilobolus	Sporulation	Period is effectively temperature independent—Uebelmesser, 1954. Temperature sensitivity also noted—Schmidle, 1951; Uebelmesser, 1954.
	Gonyaulax	Bioluminescence	Period ranges from 22.8 hours at 16°C to 26.5 hours at 26.7°C. Temperature coefficient is thus negative. Temperature sensitivity is also noted—Hastings and Sweeney, 1956.
	Paramecium	Mating reaction	Period is effectively independent of temperature with normal physiological range —Ehret, 1956.
Entrainment and frequency demultiplication (Entrainment to a periodic cycle is followed by a reversion to the natural period if the cultures are put in constant conditions)	Euglena	Phototaxis	Entrainment to LD cycles of equal duration of light and dark with periods ranging from 16 hours up to 48 hours. Frequency demultiplication by a factor of ½ observed with LD cycles of 12: 36 and 15: 33 hours of light: *dark*—Pittendrigh and Bruce, 1956.
	Oedogonium	Sporulation	Entrainment to LD cycles of 9: 9 and 12: *12* and temperature cycles of 24-hour periodicity—Buhnemann, 1955a, c.
	Pilobolus	Sporulation	Entrainment to LD cycles of 4: *4*, 6: 6, 8: *8*, 10: *10*, 12: *12*, 16: *16*, 18: *18*. Frequency demultiplication with LD cycles of 1: *47*, 2: *27*, 17: *12* (giving 24, 29 and 29-hour rhythms, respectively). Entrainment to temperature cycles of 24-hour period—Uebelmesser, 1954; and Schmidle, 1951.
	Gonyaulax	Bioluminescence	Entrainment to a 32-hour (as well as 24) cycle of light and dark—Hastings and Sweeney, 1956.
	Daldinia	Sporulation	Entrainment to a 6: 6 LD cycle—Ingold and Cox, 1955.
Single stimulus initiation and resetting	Euglena	Phototaxis	Rhythms lost in LL and rhythms lost in DD may be reinitiated by a LL to DD transition or by a single light shock (6 hours to 12 hours) respectively. A going rhythm in DD may be reset in phase by a single light shock (6 or 12 hours)—Bruce & Pittendrigh, 1956; and Pittendrigh & Bruce, 1956.

TABLE 3 (*continued*)

Single stimulus initiation and resetting (Cont'd.)	Gonyaulax	Bioluminescence	Rhythms lost in LL are reinitiated by the transition from light to dark—Sweeney & Hastings, 1956.	
	Pilobolus	Sporulation	Rhythms lost in LL are reinitiated by a light to dark transition and rhythms lost in DD are reinitiated by a 24-hour light shock. A single temperature shock will initiate a rhythm—Schmidle, 1951.	
	Oedogonium	Sporulation	Rhythms lost in DD are initiated by a dark to light transition—Buhnemann, 1955a.	
	Neurospora	"Ring formation" or zonation	A rhythm in DD is initiated by the transition from light to dark—Brandt, 1953.	
	Paramecium		Single stimulus resetting observed—Ehret, 1956.	
Conditions for persistence and loss of rhythms	Rhythms persisting in DD and lost in LL.	Paramecium	Mating reaction	Ehret, 1951
		Pilobolus	Sporulation	Schmidle, 1951
		Neurospora	Ring formation	Brandt, 1953
		Daldinia	Sporulation	Ingold & Cox, 1955
	Rhythms persisting in LL and lost in DD.	Oedogonium	Sporulation	Bühnemann, 1955a
	Special cases	Euglena	Phototaxis	These organisms are photosynthetic and require some light to get energy. In the case of Euglena, continuous light suppresses the rhythm; and in the case of Gonyaulax, the thythm will persist in low level light intensity. For further details, see the original papers.
		Gonyaulax	Bioluminescence	
Considerations of rhythms not claimed to be endogenous	Hydrodictyon	Photosynthesis and growth	Persistence in both LL and DD of apparent learning of rhythms with periods not close to 24 hours, e.g. 17½ hours—Pirson, Schön, Döring, 1954; and Schön, 1955.	
	Penicillium	Ring formation	A small temperature fluctuation [$\pm 1^{\circ}$C for Penicillium (Sagromsky, 1952, and $\pm 1\frac{1}{2}$ or 2°C for Paramecium (Kalmus, 1935)] is claimed to be necessary and sufficient to induce a rhythm.	
	Paramecium	Cell division		
Other considerations	Effect of light of different wavelengths	Oedogonium	Sporulation & Mitosis	Light of blue wavelengths (below 550 mμ) are claimed to be most efficient in inducing rhythms—Buhnemann, 1955d; Ehret, 1951; Sagromsky, 1952b.
		Paramecium	Mating reaction	
		Penicillium	Ring formation	
	Effects of drugs	Oedogonium	Sporulation	Cyanide and other drugs could not be shown to "stop the clock"—Buhnemann, 1955b.
	Rhythms with natural periods other than 24 hours	Dictyota	Sporulation	Semi-monthly period length—Hoyt, 1927.
		Strombidium (and others)	Phototaxis	Tidal (12.4 hour) period length—Faure-Fremiet, 1951.

of the cockroach, the insect clock is intimately associated with, or part of the nervous system. Harker demonstrated that motor activity in headless roaches is non rhythmic although they remain alive and active for sometime. She also demonstrated that transfer of the sub-oesophageal ganglion from a rhytmhical donor roach restores the rhythm in the headless roach. It is equally clear from the work on plants and microorganisms, however, that one need not look to the complexity of the nervous system to find the necessary or essential features of the clock.

ENDOGENOUS RHYTHMS IN MICROORGANISMS AND SIMPLE PLANTS

The universality and diversity of endogenous rhythms is still evident at the microorganism level as table 3 shows. Although diurnal rhythms have not been observed to occur in bacteria, a great variety of microorganisms, including protozoa and single-celled algae, as well as filamentous algae and fungi, exhibit diurnal periodicities.

The demonstration of an endogenous rhythm in single-celled microorganisms has only been done at the population level, and it is of course a disadvantage of such microorganisms that one must work with populations, nevertheless, insofar as there are common features of endogenous rhythms it is of the greatest importance to know that diurnal rhythms exist in single cells without a nervous system. Furthermore, it is desirable to know to what extent the rhythms in microorganisms exhibit characteristics which are common to those in other multicellular organisms. Fundamental to the whole point of view taken in this paper is the question whether the natural period can be close to, but measurably different from 24 hours. Following this is the question of temperature independence of the period of the rhythm in conditions constant with respect to light and temperature. The entrainability to cycles of light and dark and temperature cycles is another. It is also interesting to examine the diversity of conditions in which persistent rhythms are observed and to consider what features, if any, are unique to microorganisms.

Demonstration of a natural period close to, but not necessarily exactly equal to 24 hours. In most of the microorganism rhythms, a departure of the period of the rhythms from 24 hours has been demonstrated. In some cases where this has not been demonstrated to be the case, such as the sporulation rhythm in Daldinia (Ingold & Cox, 1955), for example, the eventual loss of the overt rhythm (after about two weeks) in the dark is probably due to loss of synchrony of the individual rhythms in the population.

Temperature sensitive but temperature independent. In none of the rhythms investigated has it been shown that there is a significant temperature dependence. The smallest Q_{10} reported for any of the endogenous rhythms is probably that reported by Bühnemann (1955 c) for Oedogonium, and in this case only one period length was taken as a measure of the period at different temperatures. Furthermore, the temperature coefficient

considered as the derivative of the rate with respect to the temperature in the case of Oedogonium is negative. The endogenous rhythm in *Pilobolus sphaerosporus* would appear to be essentially temperature independent from the work of Uebelmesser (1954), although some of Schmidle's (1951) data show quite clearly that the period of the rhythm need not be precisely 24 hours. Hastings and Sweeney (1956) have shown that the rhythm of bioluminescence in Gonyaulax is effectively temperature independent, as summarized in table 3, however, the small temperature coefficient is negative as is the case with Oedogonium. Bruce and Pittendrigh (1956) have shown that the rhythm of phototactic response in *Euglena gracilis* is effectively temperature independent, and Ehret (1956) has found no temperature dependence of the period length for the rhythm of mating reaction in *Paramecium bursaria.* Other investigations specifically aimed at settling the question of temperature independence do not seem to have been made; however, approximate temperature independence is implied by the fact that all of the rhythms studied have periods which are close to 24 hours independently of the arbitrary temperature chosen for the investigation. There is some suggestion, from the limited amount of data available, that the Q_{10}'s for endogenous rhythms in microorganisms, although small, tend to be larger than the Q_{10}'s of the rhythms in insects and other higher organisms. This is consistent with the idea that temperature independence is achieved by virtue of a temperature compensating mechanism which has evolved more in the case of the higher organisms. Temperature sensitivity is evidenced by the demonstrations of entrainment to temperature cycles and by the initiation of rhythms by temperature shocks (see table 3). The existence of temperature coefficients which are negative as well as positive should be pointed out for the significance with respect to speculations regarding the mechanism, whereby temperature independence is achieved.

Entrainability to periodic cycles. It has been noted that all of the rhythms are synchronized in nature to the daily 24-hour cycle. The periodic alternation of light and dark (with a 24-hour period) seems in all cases to be sufficient, though not always necessary, to induce a rhythm and this seems to be the only variable which has been shown to be effective in all cases. It is unlikely that a periodic diurnal variation in the temperature can be equally effective, but the data with respect to temperature fluctuations are less complete. It has been demonstrated that temperature fluctuations alone can induce rhythms in the following microorganisms: *Pilobolus sphaerosporus* (Schmidle, 1951 and Uebelmesser, 1954), *Oedogonium cardiacum* (Bühnemann, 1955 c), and Penicillium (Sagromsky, 1952 b). The effect of light seems to be stronger than temperature, although Sagromsky (1952 b) claims that a periodic temperature fluctuation of as little as 1° is sufficient to induce a rhythm in Penicillium; whereas, variations less than this resulted in no rhythm. Kalmus (1935) investigated the effect of constant temperature and of a small (± 1 or 2° C) temperature fluctuation on the division rate in Paramecium and noted that small temperature fluctuations of 20-hour and 24-hour period could induce a rhythm. Whether

this is an additional manifestation of the known 24-hour rhythm of mating reaction in Paramecium (Ehret, 1951); or whether it is merely an exogenously controlled synchronization of cell division, such as has been observed in many microorganisms, remains to be demonstrated.

Pilobolus (Uebelmesser, 1954), Oedogonium (Bühnemann, 1955 a, c), Gonyaulax (Hastings and Sweeney, 1956), and Euglena (Pittendrigh & Bruce, 1956), have been entrained to cycles of period length different from 24 hours using light and dark cycles. Although certain results of these experiments are peculiar to each of the above three organisms, one generalization stands out. After the entraining periodic cycle is stopped and the cultures put in constant conditions; the rhythm returns to a 24-hour period. An outstanding exception to this generalization has been found by Pirson, Schön, and Döring (1954), and Schön (1955) working with the alga Hydrodictyon. A rhythm of oxygen production (or use), as well as a rhythm in the rate of elongation of filaments is observed to be induced, by repeated 12:*12*, *6:6*, and 10½:7 hour cycles of light: *dark*. In each case, the rhythm persists with the entrained period (24, 12, or 17½ hours) for two or three cycles in both continuous light and continuous darkness. This exception seems particularly important for the bearing it may have on the evolutionary development of the biological clock. Clearly, more experiments are needed to clear up the question of whether the Hydrodictyon system might be considered as a sort of clock precursor, the true biological clock having evolved from this by organic selection (Pittendrigh, 1957; Pittendrigh and Bruce, 1957).

Effects similar to those observed in Hydrodictyon have been observed in other algae, and an observation made by Uebelmesser on Pilobolus might be mentioned in this connection though further experiments would be necessary to determine whether the effect is related. Specifically, Uebelmesser showed that the sporulation rhythm in Pilobolus may be entrained to an 8-hour rhythm by alternating exposures to four hours of light and four hours of darkness. If a culture is entrained to this cycle and then put in constant darkness, four sporulation peaks occur at 8-hour intervals before the rhythm of sporulation reverts to a 24-hour period. Speculation, concerning effects such as these should probably be deferred until more information of this type has accumulated. The possible significance of the Hydrodictyon result should not be ignored, however, by taking the view that this is a "learned rhythm" and hence a phenomenon of quite a different character than the endogenous rhythms.

Conditions for the initiation, persistence and loss of rhythms. Although in some cases endogenous rhythms persist both in constant darkness and constant light, this is not usually the case in insects or microorganisms. Continuous light more frequently supresses a rhythm than constant darkness, although this is not always the case, and it is possible that a rhythm suppressed in light of one intensity would not be suppressed in light of another intensity. It is also possible that the overt expression of a rhythm (color change for example) might be suppressed due to some physiological

factor without any interference with the basic clock. Thus the loss or absence of an overt rhythm should not be taken necessarily to imply stopping of the clock. Likewise, the initiation of an overt rhythm by a single or repeated stimulus need not imply the starting of the clock, (cf. Pittendrigh & Bruce, 1957).

If we maintain the mental reservation that initiation of population rhythms might more appropriately be described as resetting of already initiated individual rhythms, it may be said that a single stimulus is sufficient to initiate a rhythm (the phase of which is correlated with the time of occurrence of the stimulus) in several of the microorganisms. This has been shown to be the case for Pilobolus (Schmidle, 1951), Oedogonium (Bühnemann, 1955 a), Gonyaulax (Sweeney and Hastings, 1956), Euglena (Pittendrigh and Bruce, 1956), and Neurospora (Brandt, 1953), (and in confirming experiments in this laboratory).

Loss of rhythm may also be induced in cultures which have previously exhibited rhythmic behavior. Constant light seems to be the most effective means of generally accomplishing this. Pilobus (Schmidle, 1951), Eulena (Pohl, 1948), Gonyaulax (Sweeney & Hastings, 1956) Neurospora (Brandt, 1953), and Daldinia (Ingold and Cox, 1955) are microorganisms for which this has been shown to be the case. In Oedogonium (Bühnemann, 1955 a), on the other hand, a rhythm of sporulation occurs in continuous light when non-sporulating cultures in the dark are transferred to the light, and in Gonyaulax a rhythm may continue in light of low intensity but be lost in bright light (Hastings and Sweeney, 1956). The loss of rhythms in continuous light is probably dependend in general on the light intensity, and it cannot be assumed that a rhythm might not persist in very low level light intensity or light of a specific color.

Single stimuli involving light and temperature changes can induce rhythmicity in otherwise non-rhythmic organisms, though perhaps not quite as efficiently as the repeated stimuli. Thus, a single light transition (from dark to light, or in some cases light to dark) is sufficient to induce a rhythm in practically all of the microorganisms if conditions are otherwise appropriate. A single temperature transfer has been claimed to be sufficient in Pilobolus (Schmidle, 1951), and this experiment does not seem to have been tried in any other microorganism. As well as these step type stimuli, there has also been tried single stimuli of finite duration. A single exposure of a dark-grown culture to light followed by continuous darkness will start a rhythm in Pilobolus (Uebelmesser, 1954), for example, and flashes of very short duration are effective. Concerning the general question of the phase relationship of rhythms to the time of occurrence of stimuli, such as for example, the question of whether one can meaningfully speak of the phase of the rhythm as being correlated with the dawn or with sunset; it seems likely that no clear-cut generalizations can be made. As with insects, one would like to interpret factors bearing on this question from the adaptive point of view. As Pittendrigh (1954) has emphasized,

the eclosion rhythm in Drosophila must be timed from dawn, and with nocturnal insects such as the cockroach there seems to be a correlation of the phase of the rhythm to sunset. However, as one can easily see, the problem can not be so simply stated, and it should not be presumed that both factors cannot be simultaneously operating. Nevertheless, it is worth pointing out that in those cases where single transition occurs and induces a rhythm, the correlation is unique. Thus, the transition from light to dark can be thought of as setting the rhythm in Euglena (Pittendrigh and Bruce, 1956), Pilobolus (Schmidle, 1951), Neurospora (Brandt, 1953), and Gonyaulax (Sweeney and Hastings, 1956), for example, whereas the transition from dark to light sets the rhythm in Oedogonium (Bühnemann, 1955 a). Attention should be drawn to this aspect of the results of Brandt (1953) using Neurospora, which have been confirmed by recent experiments in this laboratory, because of the widespread belief that "ring formation" (e.g., Tagesringe) in fungi is entirely exogenously controlled.

Other considerations. The influence of different wavelengths on the sporulation and mitosis rhythms in Oedogenium has been investigated by Bühnemann (1955 d) with the conclusion that there is a decreasing efficiency, as regards the effect on endogenous rhythms, as the wavelength is increased from the blue to the red. The suggestion is made that only wavelengths below 550 mμ are involved in the photoreceptor system. In this respect, it is noted that Sagromsky (1952 b) found that ring formation (zonation) occurs in many of the fungi if they are exposed to alternating light and darkness when the wavelength is less than 550 mμ, but not with longer wavelengths. Ehret (1951) also obtained an action spectrum for the relative efficiency of light of different wavelengths in the mating reaction of *Paramecium bursaria* and on the basis of the higher efficiency of the shorter wavelengths suggested that a flavin acts as the photoreceptor.

Very few investigations of the effect of drugs have been made, although Bühnemann (1955 b) has shown that cyanide and other respiratory poisons do not "stop the clock" in Oedogonium. More investigations of the type Bühnemann has made are desirable to see to what extent generalizations can be made concerning the more detailed characterization of the effect of factors such as different wavelengths, respiratory poisons, and drugs of other types. Interpretations of results of this type are of course highly speculative, and one is always up against the problem of how many "black boxes" lie between the experimenter and the "clock". It is for this reason that we feel that the comparative approach has merits, and it is reassuring to find that the rhythms in microorganisms have characteristics similar to those in insects and other organisms. When one's interest is in the clock itself rather than in some other aspect, the above point of view would be well exemplified by considering the difficulties which one would encounter in using Hydrodictyon as a basis for one's ideas about the possible mechanism of the clock.

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A CYTOLOGICAL STUDY OF THE SWEET POTATO PLANT IPOMOEA BATATAS (L.) LAM. AND ITS RELATED SPECIES

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INTRODUCTION

Millions of people are dependent upon the cultivation of the sweet potato for subsistence, and it ranks as one of the most important world food crops. Despite this, research along cytological lines on this crop and its related species has been much neglected. Chromosome numbers of many species of the genus Ipomoea have not yet been determined, and few varieties of commercial sweet potatoes have been cytologically analyzed. The superior pioneer works of Kano (1929), Walcott (1937), King and Bamford (1937), and Rao (1947) have provided much valuable information concerning chromosome numbers to research staffs working on sweet potato improvement, but more is needed. It is astonishing that the first meiotic study of the sweet potato itself was not published until 1953 (Ting and Kehr, 1953).

The present study was undertaken with the objective of examining the meiotic features in various varieties of the sweet potato plant, and determining the chromosome numbers of the other species in the genus Ipomoea. Particular attention was paid to those species which are taxonomically closely placed to the sweet potato plant. In addition, an interspecific hybridization program was initiated in order to clarify the phylogenetic relationship between the sweet potato plant and the other species in the genus.

MATERIALS AND METHODS

Varieties of the sweet potato plants used in this study were from the sweet potato breeding plot of the Department of Horticulture, Louisiana State University at Baton Rouge. Seeds and vines of the other Ipomoea species were obtained mainly from West Indies and the other parts of the world.

Flower buds were collected at 5:00 A.M., since it was learned that meioses in the sweet potato plants mostly occurred at about one hour before daybreak (Ting and Kehr, 1953). Freshly collected materials were immediately placed in an alcohol-chloroform-acetic fixative which was prepared only a few minutes before use. The aceto-carmine smear technique was followed throughout this study.

The drawing was made with the aid of a camera lucida utilizing a 60x apochromatic objective and a 30x compensating eye piece, to give the magni-

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fication as indicated on the plate. The photomicrograph was produced from the negative obtained by using a versatile Bausch and Lomb photomicrographic attachment.

Interspecific hybridizations were attempted in 1952, 1953 and 1954. Pollinations were done in both the greenhouse and the breeding nursery at Baton Rouge. The time of pollination was from 5 to 8 o'clock in the morning.

RESULTS

1. *Meiosis in different varieties of the sweet potato plant:* Most of the meiotic stages of the sweet potato plant were found and analyzed, including pachytene, diplotene, diakinesis, metaphase I, anaphase I, telophase I; second prophase, metaphase, anaphase, telophase and quartet stage.

TABLE 1

THE NUMBER OF BIVALENT CHROMOSOMES AND THE AVERAGE NUMBER OF SECONDARY ASSOCIATION OF BIVALENTS (BASED ON 11-27 CELLS) AND CHROMOSOME IRREGULARITIES IN EIGHT VARIETIES OF THE SWEET POTATO PLANT.

Variety	No. of bivalents	Chromosome irregularities	Average number of secondary association of bivalents in a single cell		
			Group of 2 bivalents	Group of 3 bivalents	Group of 4 bivalents
L21	45	none	5.3	6	none
LO-87	45	none	5	5	none
LO-123	45	none	6.3	4	none
LO-99	45	none	7	3.4	none
L-130	45	none	9	5	none
LO-74	45	none	7	3	none
Oklahoma 24	45	none	5	6	Occasionally found
L9-32*	45	Univalents, laggards	7	3	none

*Based on Ting and Kehr's data, 1953.

Chromosome numbers were determined at metaphase I of meiosis of the following varieties: L21, LO-87, LO-123, LO-99, L-130, LO-74 and Oklahoma 24. It was found that all seven varieties had n = 45 chromosomes (table I). No aneuploid cells were ever observed.

It was of particular interest to see that in polar view of metaphase I, secondary association of bivalent chromosomes was found as a consistent phenomenon in all the plants. Those associated bivalents were somewhat similar in size and shape (Fig. 1). Some of these bivalents were connected by a strand of light chromatic substance indicating probable affinity of such bivalent chromosomes. The number of two bivalents associated together varied from five to nine; that of three bivalents, from three to six. The frequency of four bivalents associated together was found extremely low (table I).

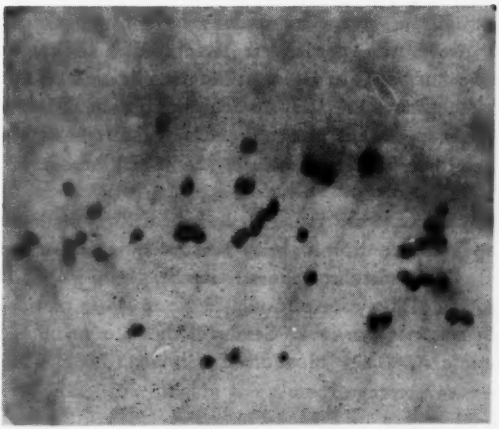

FIGURE 1. Photomicrograph. Variety LO-87, showing five groups of two bivalents, five groups of three bivalents and one group of four bivalents, associated together. The total number of bivalents is 45. Metaphase I, polar view. 1850x.

Chromosome irregularities, such as laggards, ring-chromosomes, chromatid bridges, fragments and univalent chromosomes were not found, except in the variety L9-32. In this variety, in addition to laggards, univalent chromosomes at metaphase I were frequently noticed, the number of which varied from 18 to 30, based on a limited number of cells studied. In general the univalent chromosomes appeared lighter in staining and smaller in size than those of the normal bivalent chromosomes (Fig. 2).

2. *Chromosome numbers in the other Ipomoea species:* By making counts at metaphase I or anaphase I of meiosis, chromosome numbers of seven Ipomoea species were determined for the first time, including some of the species that have been frequently discussed in the literature dealing with the evolution of the sweet potato plant. In table 2, both the chromosome numbers of these species and the sources of seeds or plants are recorded. It is apparent, in this table, that both *Ipomoea gracilis* (*I. fastigata*) and *I.*

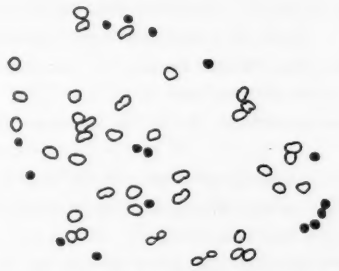

FIGURE 2. Camera lucida drawing. Variety L9-32, showing sixteen univalent chromosomes (solid) and thirty-seven bivalent chromosomes (outline). Metaphase I, polar view. 1700x.

tiliacea have n = 30 chromosomes, or they have 60 somatic chromosomes. In other words, these two species are tetraploid if 15 is accepted as the basic chromosome number in the genus. Seeds of *I. gracilis* were collected from Cuba, and the vines of *I. tiliacea* were obtained from Puerto Rico. Both were grown in the sweet potato breeding plot at Baton Rouge, Louisiana. Neither species produced storage roots, but both produced a profusion of flowers and set seeds well.

Five Ipomoea species were found to have n = 15 chromosomes (table 2). Hence, these species are diploid, with 30 somatic chromosomes. The size and shape of the bivalent chromosomes in the diploid species were found very similar to those in the tetraploid species. Most of the chromosomes appeared rounded at metaphase I. Seeds of these were obtained from West Indies and Louisiana. They blossomed very readily at Baton Rouge, but none of them set any storage roots.

TABLE 2

CHROMOSOME NUMBERS FOUND AT METAPHASE I OR ANAPHASE I OF MEIOSIS AND SOURCES OF SEEDS AND PLANTS OF SEVEN IPOMOEA SPECIES.

Species	Chromosome No. (n)	Sources of seeds or plants
Ipomoea gracilis R. Br. (*I. fastigiata Sweet*)	30	Cuba
I. tiliacea (Willd.) Choisy	30	Puerto Rico
I. asarifolia (Desr.) Roem. & Schult.	15	Cuba
I. carnea Jacq.	15	Puerto Rico
I. arborescens G. Don	15	Cuba
I. pendula Choisy	15	Louisiana
I. quinquefolia L.	15	Cuba

In addition to the above species, two other species: *Ipomoea hederacea* (L.) Jacq. and *I. learii* Paxton, of which the chromosome numbers at metaphase I were likewise counted. Prior to this study, chromosome numbers in the somatic cells of these two species were reported to be 30 (King & Bamford, 1937). During the present study, the numbers of bivalent chromosomes at metaphase I were determined to be 15. This study therefore confirms the previous determinations of the chromosome numbers in these two species.

Because of the limited amount of time available, the phenomenon of secondary association of bivalent chromosomes at metaphase I of the tetraploid and diploid species was not studied.

3. *Interspecific hybridization:* In order to find out the phylogenetic relation of the sweet potato plant with the other species in the genus Ipomoea, interspecific hybridizations were made. A total of about 3000 crosses were attempted during 1952, 1953 and 1954. The following species were

used as male parents in crosses with the sweet potato plant: *Ipomoea asarifolia* (Desr.) Roem. & Schult., *I. bona-nox* L., *I. carnea* Jacq., *I. arborescens* G. Don, *I. dissecta* (Jacq.) Pursh, *I. gracilis* R. Br., *I. grandiflora* Jacq., *I. hederacea* (L.) Jacq., *I. learii* Paxton, *I. muricata* Cav., *I. pandurata* L., *I. pendula* Choisy, *I. pescaprae* (L.) Sweet, *I. pubescens* Lam., *I. polyanthes* Roem. & Schult., *I. purpurea* (L.) Lam., *I. quinquefolia* L., *I. ruber* var. *palustris* Urban, *I. rubro-caerulea* Koen., *I. setosa* Ker, *I. sinuata* Orteg. *I. tiliacea* (Willd.) *Choisy, I. tricolor* Cav., *I. triloba* L.

When the sweet potato plant was used as pollen parent, pollinations were made with the following species: *Ipomoea asarifolia* (Desr.) Roem. & Schult., *I. carnea* Jacq., *I. arborescens* G. Don, *I. gracilis* R. Br., *I. hederacea* (L.) Jacq., *I. pescaprae* (L.) Sweet. *I. rubro-caerulea* Koen., *I. sinuata* Orteg., *I. tiliacea* (Willd.) Choisy.

The numbers of pollinations in the above crosses between any two species varied from 2 to 630. Because of the fact that practically all of the crosses were unsuccessful, the exact number of pollinations between any two species is not given here. The largest numbers of pollinations were those between *Ipomoea batatas* and *I. tiliacea,* and between *I. batatas* and *I. gracilis;* of which the numbers of pollinations are 630 and 161 respectively.

Despite the many pollinations made in two directions, only in the cross *Ipomoea batatas* × *I. pescaprae,* did there seem to be any stimulation of the ovules of the sweet potato plant. Two non-viable seeds were harvested from this cross. However, no true interspecific hybrid F_1 plants involving the sweet potato as one parent were obtained from any combinations among the crosses.

DISCUSSION

House (1908) postulated that the modern sweet potato plant was derived from the tropical American plant, *I. tiliacea,* by a means of cultivation. This hypothesis was later supported by other botanists.

Cytological studies of *I. batatas* and *I. tiliacea* clearly demonstrate that the sweet potato plant could not have been originated from *I. tiliacea* by cultivation *per se.* Chromosome numbers of all sweet potato varieties studied to date have consistently been determined as $2N = 90$, or hexaploids. On the other hand *I. tiliacea* has 60 somatic chromosomes, and it is tetraploid. Furthermore, there had been not a single cross made between these two species, no matter in which direction the crosses were attempted. The complete absence of similarity in their chromosome numbers and the lack of fruitful crosses between these two species, perhaps, constitute the most convincing evidence that the modern sweet potato plant could not have originated directly from *I. tiliacea* merely by cultivation. On the basis of these studies the authors maintain that House's theory on the origin of the sweet potato plant should be repudiated.

Meiotic studies on the different varieties of the sweet potato plant are indicative that the sweet potato plant is probably an amphidiploid. It was

consistently found that the chromosomes in the sweet potato plant behaved differently from those in an auto-polyploid plant or a normal diploid plant in that they demonstrated the phenomenon of secondary association of bivalent chromosomes, and they did not form multivalent associations in polar view of metaphase I. In tracing the evolutionary nature of a plant, multivalent associations of bivalent chromosomes may be used as one of the criteria to distinguish autoploid from alloploid. The phenomenon of secondary association of bivalent chromosomes at metaphase I suggests that the genomes involved in making up the sweet potato plant did not have enough similarity to form multivalents, but they did have enough homology to manifest the unusual phenomenon of secondary pairings. Chromosome studies of the genus Ipomoea would indicate that one of its ancestral species has a chromosome number of 60, the other has a chromosome number of 30. By natural doubling the chromosome number of the F_1 hybrid between these two ancestral species, the amphidiploid plant, or the prototype of modern sweet potato plant, came into being. However, the two hypothetic ancestral species of the modern sweet potato plant are still unknown. Although there were more than 3000 interspecific crosses attempted using both diploid and tetraploid species, none of the crosses was successful. The cross *Ipomoea batatas* (L.) Lam. × *I. pescaprae* (L.) Sweet did produce two non-viable seeds, but it seems unlikely that *I. pescaprae* is one of the ancestral species.

Secondary association of bivalents at metaphase I, probably signifies that the diploidization process in the sweet potato plant has not yet been completed. In other words, the sweet potato plant is a raw alloploid species. From the cytogenetic point of view, a raw alloploid species should become progressively diploidized as time advances until the chromosome behavior resembles that in a normal diploid species (Stebbins, 1947). Hence, it appears that the sweet potato plant is a relatively recent species.

CONCLUSIONS AND SUMMARY

Chromosome members of seven varieties of the sweet potato plant *Ipomoea batatas* (L.) Lam. were counted for the first time to be n = 45. Secondary association of bivalent chromosomes at metaphase I was observed in all of the varieties studied. The secondary association of bivalent chromosomes is considered as an indication that the modern sweet potato plant is an amphidiploid which probably originated as a combination between a diploid species and a tetraploid species and that diploidization in the sweet potato plant has not yet been completed.

Chromosome numbers of seven other American species in the genus Ipomoea were also counted for the first time. Two of the seven species are tetraploid with a gametic chromosome number of 30; the rest are diploid with a gametic chromosome number of 15.

Because of the difference in the chromosome numbers between the sweet potato plant and the species *Ipomoea tiliacia* (Willd.) Choisy, the theory

that the modern sweet potato plant originated from *I. tiliacea* by cultivation must be rejected.

Although there were more than 3000 interspecific crosses attempted between the sweet potato plant and the other tetraploid and diploid species in the genus Ipomoea, not a single viable F_1 hybrid plant was obtained. The search for the ancestral species of the modern sweet potato needs to be further explored.

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THE AMERICAN SOCIETY OF NATURALISTS
SECRETARY'S REPORT, 1956

The annual business meeting of the Society was held in Storrs, Connecticut on the campus of the University of Connecticut in connection with the AIBS on August 29, 1956 with President E. Newton Harvey presiding.

The Secretary's report of the last meeting was accepted as it appears in the March-April 1956 issue of the American Naturalist.

The report of the Nominating Committee (Paul C. Mangelsdorf, Chairman, Marston Bates, and G. Ledyard Stebbins) was presented by the Secretary. With no further nominations from the floor, the following officers were unanimously elected:

President (1957)—William C. Steere, Stanford University

Vice President (1957)—I. Michael Lerner
University of California, Berkeley

Treasurer (1957-59)—George F. Sprague, Iowa State College

A motion was made an unanimously passed to hold the next annual meeting with AIBS at Stanford University in August 1957.

Sixteen persons, nominated by members and approved by the Executive Committee, were elected to membership. A list of those elected who have accepted membership at the present time follows:

Relph W. Dexter, Kent State University, Kent, Ohio.
Vincent Groupé, Rutgers University, New Brunswick, N. J.
T. C. Hsu, M. D. Anderson Hospital, Houston, Texas
Clark Hubbs, University of Texas, Austin, Texas
Thomas King, Lankenau Hospital, Philadelphia, Pa.
Robert C. King, Northwestern University, Evanston, Ill.
R. C. Lewontin, N. C. State College, Raleigh, N. C.
M. Y. Menzel, Tallahassee, Fla.
Montrose J. Moses, Rockefeller Institute, New York, N. Y.
H. F. Robinson, N. C. State College, Raleigh, N. C.
E. B. Spiess, University of Pittsburgh, Pittsburgh, Pa.
Ray L. Waterman, Northwestern University, Evanston, Ill.
Charles Yanofsky, Western Reserve University, Cleveland, Ohio

The treasurer's report was read by the Secretary and accepted.

The Secretary made a few remarks concerning the possibility that the Society might operate a "coffee bar" rather than attempt to renew the annual banquet. It appears that most campuses have student snack bars; a coffee bar would be superfluous under these circumstances.

The Secretary also reported on deaths of members during the past year. A list of members who have died within the past year follows:

Leroy R. Abrams, Stanford University
C. C. Adams, Albany, N. Y.
Mark H. Adams, New York University
Samuel Brody, University of Missouri
C. T. Brues, Harvard University
A. J. Carlson, University of Chicago
Roy E. Clausen, University of California
H. E. Crampton, American Museum of Natural History
B. M. Duggar, Lederle Laboratories, Pearl River, N. Y.
W. W. Garner, U. S. Department of Agriculture, Beltsville, Md.
E. M. Gilbert, University of Wisconsin
F. R. Jones, University of Wisconsin
T. H. Kearney, California Academy of Science
A. C. Kinsey, Indiana University
H. R. Kraybill, American Meat Institution Foundation, Chicago, Ill.
C. F. W. McClure, Princeton University
N. E. McIndoo, U. S. Department of Agriculture, Beltsville, Md.
E. D. Merrill, Arnold Arboretum, Boston, Mass.
G. T. Moore, Missouri Botanical Garden
G. M. Reed, Pittsburgh, Pa.
C. O. Rosendahl, University of Minnesota
J. W. Scott, University of Wyoming
C. L. Shear, Monroe, La.
Waldo Shumway, Stevens Institute of Technology, Hoboken, N. J.
Charles Thom, Port Jefferson, N. Y.
R. M. Yerkes, Yale School of Medicine

Professor L. C. Dunn, Editor of the American Naturalist, reported on the status of that journal; his report was accepted. The Executive Committee in consultation with Professor Dunn, appointed the following persons to the Editorial Board of the American Naturalist (Class of 1959):

Jack Schultz	K. V. Thimann
G. L. Stebbins	C. S. Pittendrigh

A motion of thanks to Dr. Richard Goodwin, the Society's Local Representative at the Storrs meetings, to AIBS, and to the University of Connecticut, was passed unanimously.

President Harvey has appointed S. Meryl Rose to the AAAS Council for the years 1957-58. Clark Dalton has been appointed to the AIBS Governing Board to replace C. G. Huff.

The following persons have been appointed as the Nominating Committee for 1957 by President Harvey:

Ralph E. Cleland, Chairman Th. Dobzhansky T. M. Sonneborn

The Society's program at the Storrs meetings consisted of the following:

August 28. Presidential Address: E. Newton Harvey. Evolution of bioluminescence.

August 29. Symposium: Biological chronometry.
Brown, F. A. The general nature and significance of the problem.
Stephens, G. C. Twenty-four hour rhythmicity in marine organisms.
Pittendrigh, C. S. and V. G. Bruce. Cellular clocks in insects and microorganisms.
Folk, G. E. Twenty-four hour rhythms of mammals in a cold environment.
Fingerman, M. Lunar rhythmicity in marine organisms.

The Biological Laboratory
Cold Spring Harbor, N. Y.

Bruce Wallace, Secretary

REPORT OF TREASURER

AMERICAN SOCIETY OF NATURALISTS

Balance on hand, First National Bank, Baltimore, Aug. 15, 1955	$1216.87
Dues and subscriptions: Aug. 15, 1955 to Aug. 1, 1956	2898.90
TOTAL RECEIPTS	$4115.77
EXPENDITURES	
Society expenses:	
Stamps (Treasurer)	15.00
Addressographing	2.96
Secretarial expenses 1953-56 (W. P. Spencer)	64.79
Secretarial expenses 1955-56 (Bruce Wallace)	93.32
Travel: AIBS meetings, East Lansing, Mich., 1955	
Treasurer (C. P. Swanson)	90.00
Secretary (W. P. Spencer)	25.50
AIBS membership	513.00
L. C. Dunn, editorial assistance	300.00
Science Press, 541 subscriptions	1893.50
Bank charges	1.58
TOTAL EXPENSES	$2999.65
Balance on hand, First National Bank, Baltimore, Aug. 1, 1956	$1116.12

C. P. Swanson, Treasurer

Audited and found to be correct. August 28, 1956
Karl Sax; Alan D. Loyn

REPORT OF THE EDITOR

During the period September 1, 1955 to August 31, 1956, 73 manuscripts were received of which 21 were rejected. The fate of the manuscripts, by classes, reveals the direction in which we have been moving. The manuscripts accepted concern a wider variety of biological problems than in previous years.

	Received	Rejected
Data papers	27	16
General papers	15	3
Symposium papers	8	....
Letters	19	....
Methods	3	2
Supplements	1	....

During this period we published 6 issues containing 30 papers, 11 letters to the editor, 20 pages of book notices, and 4 pages of reports of the Society. By decision of the editorial board publication of book notices was discontinued with the issue of September 1956, and it was decided that due to the present influx of manuscripts we should have to apply more strictly our policies of preference for symposium papers and those of general interest in which synthesis and interpretation are predominant. The journal is now being issued on time, and we propose to accept only as many manuscripts, apart from symposium papers, as can be printed within 6 to 8 months of receipt.

L. C. Dunn